PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 :
C12N 15/31, C07K 14/315, A61K 39/09 // C12N 15/62

A1

(11) International Publication Number: WO 96/23886

(43) International Publication Date: 8 August 1996 (08.08.96)

(21) International Application Number: PCT/GB96/00207

(22) International Filing Date: 31 January 1996 (31.01.96)

(30) Priority Data:
9501826.3 31 January 1995 (31.01.95) GB

(71) Applicant *(for all designated States except US)*: THE COUNCIL OF GOVERNORS OF THE UNITED MEDICAL & DENTAL SCHOOLS OF GUY'S & ST. THOMAS HOSPITALS [GB/GB]; London Bridge, London SE1 9RT (GB).

(72) Inventors; and

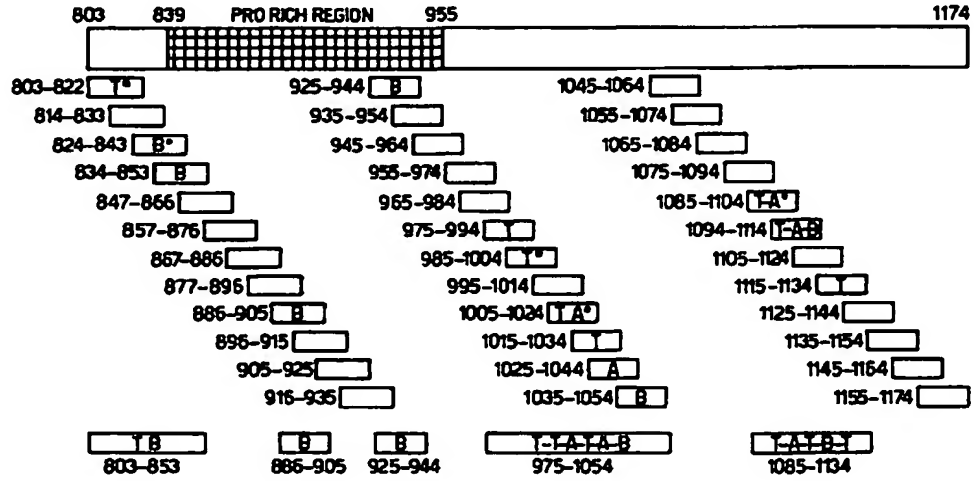
(75) Inventors/Applicants *(for US only)*: LEHNER, Thomas [GB/GB]; Guy's Hospital, Guy's Tower floor 28, London SE1 9RT (GB). KELLY, Charles [GB/GB]; Guy's Hospital, Guy's Tower floor 28, London SE1 9RT (GB).

(74) Agents: GOLDIN, Douglas, Michael et al.; J.A. Kemp & Co., 14 South Square, Gray's Inn, London WC1R 5LX (GB).

(81) Designated States: AL, AM, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, ARIPO patent (KE, LS, MW, SD, SZ, UG), Eurasian patent (AZ, BY, KG, KZ, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: POLYPEPTIDE FRAGMENTS CAPABLE OF COMPETITION WITH STREPTOCOCCUS MUTANS ANTIGEN I/II



(57) Abstract

A nucleic acid sequence which codes upon expression in a prokaryotic or eukaryotic host cell for a polypeptide product having one or more properties selected from (i) the ability to adhere to a mammalian tooth in a competitive manner with naturally occurring *Streptococcus mutans* antigen I/II, thus preventing or diminishing the adhesion of *S.mutans* to the tooth; (ii) the ability to stimulate a T-cell response; and (iii) the ability to stimulate a B-cell response, said nucleic acid sequence being selected from: (a) the sequences shown in SEQ. ID. No. 12 to 22 or the complementary strands thereof; (b) nucleic acid sequences having a length of not more than 1000 base pairs which hybridise to the sequences defined in (a) over at least 70 % of their length; (c) nucleic acid sequences having a length of not more than 1000 base pairs which, but for the degeneracy of the genetic code, would hybridise to the nucleic acid sequences defined in (a) or (b) over at least 70 % of their length and which sequences code for polypeptides having the same amino acid sequence.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AM	Armenia	**GB**	United Kingdom	**MW**	Malawi
AT	Austria	**GE**	Georgia	**MX**	Mexico
AU	Australia	**GN**	Guinea	**NE**	Niger
BB	Barbados	**GR**	Greece	**NL**	Netherlands
BE	Belgium	**HU**	Hungary	**NO**	Norway
BF	Burkina Faso	**IE**	Ireland	**NZ**	New Zealand
BG	Bulgaria	**IT**	Italy	**PL**	Poland
BJ	Benin	**JP**	Japan	**PT**	Portugal
BR	Brazil	**KE**	Kenya	**RO**	Romania
BY	Belarus	**KG**	Kyrgystan	**RU**	Russian Federation
CA	Canada	**KP**	Democratic People's Republic of Korea	**SD**	Sudan
CF	Central African Republic			**SE**	Sweden
CG	Congo	**KR**	Republic of Korea	**SG**	Singapore
CH	Switzerland	**KZ**	Kazakhstan	**SI**	Slovenia
CI	Côte d'Ivoire	**LI**	Liechtenstein	**SK**	Slovakia
CM	Cameroon	**LK**	Sri Lanka	**SN**	Senegal
CN	China	**LR**	Liberia	**SZ**	Swaziland
CS	Czechoslovakia	**LT**	Lithuania	**TD**	Chad
CZ	Czech Republic	**LU**	Luxembourg	**TG**	Togo
DE	Germany	**LV**	Latvia	**TJ**	Tajikistan
DK	Denmark	**MC**	Monaco	**TT**	Trinidad and Tobago
EE	Estonia	**MD**	Republic of Moldova	**UA**	Ukraine
ES	Spain	**MG**	Madagascar	**UG**	Uganda
FI	Finland	**ML**	Mali	**US**	United States of America
FR	France	**MN**	Mongolia	**UZ**	Uzbekistan
GA	Gabon	**MR**	Mauritania	**VN**	Viet Nam

POLYPEPTIDE FRAGMENTS CAPABLE OF COMPETITION WITH STREPTOCOCCUS MUTANS ANTIGEN I/II

This invention relates to polypeptide fragments of the Streptococcus mutans I/II antigen that are useful in treating and preventing dental caries.

Streptococcus mutans is the main etiological agent of dental caries, a disease which affects mammals including humans.

The S. mutans I/II antigen (SA I/II) is a cell surface protein with an M_r of about 185kDa. It is believed to comprise several antigenic epitopes and to be at least partly responsible for S. mutans adhesion to teeth.

SA I/II is described in British Patent No. 2,060,647, as are number antibodies to it. A putative 3.5 to 4.5 kDa fragment of SA I/II, "antigen X", has also been described in European Patent No. 0 116 472.

However, it has now become clear that "antigen X" is not a fragment of SA I/II at all. Rather, it is a separate protein that merely co-purifies with SA I/II. It is believed to be encoded by a separate gene.

Two large fragments of SA I/II, an N-terminal fragment (residues 39 to 481) and a 40kDa central fragment (residues 816 to 1213) are recognised by human serum antibodies. Within the central fragment, 80% of the sera tested recognise elements within a proline-rich region (residues 839-955) that comprises three tandem repeats. This suggests that this region includes one or more B-cell epitopes. The central fragment (residues 816-1213) is also believed to comprise one or more adhesion sites that mediate S. mutans' attachment to the tooth.

The aim of the above-mentioned work has been the development of vaccines for immunisation against dental caries. However, precise identification of the antigenic epitopes within SA I/II is a prerequisite for designing

synthetic vaccines based on it. Similarly, precise identification of adhesion sites is essential for the design of drugs against dental caries that rely on inhibiting S. mutans' adhesion to the tooth.

No antigenic epitopes (T-cell or B-cell epitopes) or adhesion sites within SA I/II have been characterised, nor has the precise location of any such regions been suggested. Also, there has been no indication of the location of S. mutans' T-cell epitopes as the above-mentioned work has concentrated on S. mutans' ability to adhere to teeth and to generate a B-cell response.

The inventors have identified a number of T-cell epitopes, B-cell epitopes and adhesion sites within residues 803 to 1114 of SA I/II. Some of the T-cell and B-cell epitopes overlap or are contiguous with each other and/or with one or more of the adhesion sites.

The presence of a number of antigenic epitopes of both types and a number of adhesion sites within the same region of SA I/II could not have been predicted and the finding that some of the adhesion sites and epitopes overlap or are contiguous with each other is particularly surprising.

These findings make it possible to design effective synthetic vaccines against dental caries as well as drugs that engender resistance against the disease or alleviate pre-existing cases of it by preventing S. mutans' adhesion to the tooth. Further, the surprising finding that some of the T-antigenic epitopes and the adhesion site are contiguous or overlapping makes it possible to design bifunctional drugs that effect immunisation against dental caries as well as preventing adhesion of S. mutans to the tooth.

Accordingly, the present invention provides a nucleic acid sequence which codes upon expression in a prokaryoic

or eukaryotic host cell for a polypeptide product having one or more properties selected from (i) the ability to adhere to a mammalian tooth in a competitive manner with naturally occurring Streptococcus mutans antigen I/II, thus preventing or diminishing the adhesion of S.mutans to the tooth; (ii) the ability to stimulate a T-cell response; and (iii) the ability to stimulate a B-cell response, said nucleic acid sequence being selected from:

(a) the sequences shown in SEQ. ID. Nos. 12 to 22 or the complementary strands thereof;

(b) nucleic acid sequences having a length of not more than 1000 base pairs which hybridise to the sequences defined in (a) over at least 70% of their length;

(c) nucleic acid sequences having a length of not more than 1000 base pairs which, but for the degeneracy of the genetic code, would hybridise to the nucleic acid sequences defined in (a) or (b) over at least 70% of their length and which sequences code for polypeptides having the same amino acid sequence code, would hybridise to the nucleic acid sequences defined in (a) or (b) over at least 70% of their length and which sequences code for polypeptides having the same amino acid sequence.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1. Depiction of the panel of overlapping 20mers used to map T-cell, B-cell and adhesion epitopes within SA I/II.

Figure 2. Proliferative responses to overlapping synthetic peptides (20^{ers}) of SA I/II.

a/ Mean S.I. ($\pm$sem) of PBMC from 30 subjects. Mean cpm with medium only was 538$\pm$112.

b) Frequency of positive responses (S.I.≥3.0, cpm>500).

Figure 3. MHC class II dependency of proliferative responses to SA I/II.

Figure 4. Serum recognition of SA I/II and recombinant polypeptide fragments. Western blots from 3 subjects are shown (panels a-c) together with rabbit anti-SA I/II antiserum (panel d). Lanes, 1, SA I/II; lane 5, recombinant 984-1161.

Figure 5. Human serum recognition of synthetic peptides of SA I/II.

a) Titres were determined by ELISA in 22 subjects to selected peptides of SA I/II and an irrelevant control peptide from SIVp27(SIV). The frequencies of sera binding the peptides with a titre >mean+2S.D. the control peptide are also indicated.

Figure 6. Inhibition of adhesion of S. mutans.

a) SA I/II and recombinant fragment 984-1161.

b) Synthetic peptides.

Figure 7. Proliferative responses of murine splenocytes following immunization with recombinant 975-1044 (SEQ. ID. No. 8)

Figure 8. Competitive inhibition of SA I/II binding by various polypeptides.

Figure 9. Dependence of competitive inhibition of SA I/II binding on concentration of two peptides.

Figure 10. Effects of substitution of certain residues on competitive inhibition.

Figure 11. Comparison of various recombinant polypeptides with respect to binding.

The polypeptides of the invention have one or more of the following properties. Firstly, they may have the ability to adhere to a mammalian tooth in a competitive manner with naturally occurring Streptococcus mutans antigen I/II, thus preventing or diminishing the adhesion of S. mutans to the tooth. Some of the peptides of the invention have been shown to inhibit adhesion of S. mutans to a tooth surface model (whole human saliva adsorbed to the wells of polystyrene microtitre plates or hydroxyapatite beads). Thus, these peptides comprise one or more adhesion sites and will adhere to a mammalian tooth in a competitive manner with naturally occurring SA I/II. Therefore, peptides according to the invention that comprise the adhesion site prevent or diminish the adhesion of S. mutans to the tooth. Peptides of the invention that comprise one or more adhesion epitopes include SEQ. ID. Nos. 1 to 6 and 8 to 10.

Secondly, peptides according to the invention may have the ability to stimulate a T-cell response. The inventors have shown that residues 803 to 854 and 925 to 1114 of SA I/II comprise a number of T-cell epitopes that are at least partially responsible for the T-cell response stimulated by the intact protein. Therefore, peptides according to the invention that comprise one or more of these the T-cell epitopes stimulate a T-cell response against S. mutans infection. Peptides of the invention that stimulate a T-cell response include those shown in SEQ ID Nos. 1 to 11.

Thirdly, the peptides of the invention may stimulate a B-cell response. The inventors have shown that residues 803 to 854 and 925 to 1114 of SA I/II comprise a number of B-cell epitopes and polypeptides according to the invention that comprise one or more B-cell epitopes stimulate a B-cell response against S. mutans infection. Peptides of the invention that comprise one or more B-cell epitopes include those shown in SEQ. ID. Nos. 1, 3 to 7 and 10.

The nucleic acid sequences of the present invention are preferably DNA, though they may be RNA. It will be obvious to those of skill in the art that, in RNA sequences according to the invention, the T residues shown in SEQ. ID. Nos. 12 to 22 will be replaced by U. Nucleic acid sequences of the invention will typically be in isolated or substantially isolated form. For example up to 80, up to 90, up to 95 or up to 100% of the nucleic acid material in a preparation of a nucleic acid of the invention will typically be nucleic acid according to the invention.

Some preferred nucleic acid sequences of the invention are those shown in SEQ. ID. Nos. 12 to 22. However, the nucleic acid sequences of the present invention are not limited to these sequences. Rather, the sequences of the invention include sequences that are closely related to these sequences and that encode a polypeptide having at least one of the biological properties of naturally occurring SA I/II. These sequences may be prepared by altering those of SEQ ID Nos. 12 to 22 by any conventional method, or isolated from any organism or made synthetically. Such alterations, isolations or syntheses may be performed by any conventional method, for example by the methods of Sambrook et al (Molecular Cloning: A Laboratory Manual; 1989)

For example, the sequences of the invention include sequences that are capable of selective hybridisation to

those of SEQ. ID. Nos. 12 to 22 or the complementary strands thereof and that encode a polypeptide having one or more of the properties defined above. Such sequences capable of selectively hybridizing to the DNA of SEQ. ID. Nos. 12 to 22 will generally be at least 70%, preferably at least 80 or 90% and more preferably at least 95% homologous to the DNA of SEQ. ID. Nos. 12 to 22 over a region of at least 10, preferably at least 20, 30, 40, 50 or more contiguous nucleotides.

Such sequences that hybridise to those shown in SEQ. ID. Nos. 12 to 22 will typically be of similar size to them, though they may be longer or shorter. However, if they are longer, they may not simply encode large fragments of native SA I/II amino acid sequence. Thus, sequences that hybridise to those of SEQ. ID. Nos. 12 to 22 may be sequences of up to 1000 bases in length, for example up to 950 or up to 933 bases in length, 933 bases being the length of the DNA sequence encoding the largest specifically identified peptide of the invention (SEQ. ID. No. 21). Also, sequences that hybridise to those of SEQ. ID. Nos. 12 to 22 must do so over at least 50% of their length, for example up to 60%, up to 70%, up to 80%, up to 90%, up to 95%, or up to 99% of their length.

Such hybridisation may be carried out under any suitable conditions known in the art (see Sambrook et al (1989): Molecular Cloning: A Laboratory Manual). For example, if high stringency is required, suitable conditions include 0.2 x SSC at 60°C. If lower stringency is required, suitable conditions include 2 x SSC at 60°C.

Also included within the scope of the invention are sequences that differ from those defined above because of the degeneracy of the genetic code and encode the same polypeptide having one or more of the properties defined above, namely the polypeptide of SEQ. ID. Nos. 1 to 11 or

a polypeptide related to one of these polypeptides in any of the ways defined below.

Thus, the nucleic acid sequences of the invention include sequences which, but for the degeneracy of the genetic code, would hybridise to those shown in SEQ. ID. Nos. 12 to 22 or the complementary strands thereof. However, such sequences may not simply encode large fragments of native SA I/II amino acid sequences. Thus, these sequences may be up to 1000 bases in length, for example up to 950 or 933 bases in length. Also, their sequence must be such that, but of the degeneracy of the genetic code, they would hybridise to a sequence as shown in SEQ. ID. Nos. 12 to 22 over at least 50% of their length, for example, up to 60%, up to 70%, up to 80%, up to 90%, up to 95% or up to 99% of their length.

Also, the nucleic acid sequences of the invention include the complementary strands of the sequences defined above, for example the complementary strands of the nucleic acid sequences shown in SEQ. ID. Nos. 12 to 22.

Nucleic acid sequences of the invention will preferably be at least 30 bases in length, for example up to 50, up to 100, up to 200, up to 300, up to 400, up to 500, up to 600, up to 800 or up to 1000 bases.

Nucleic acid sequences of the invention may be extended at either or both of the 5' and 3' ends. Such extensions may be of any length. For example, an extension may comprise up to 10, up to 20, up to 50, up to 100, up to 200 or up to 500 or more nucleic acids. A 5' extension may have any sequence apart from that which is immediately 5' to the sequence of the invention (or the native sequence from which it is derived) in native SA I/II. A 3' extension may have any sequence apart from that which is 3' to the sequence of SEQ. ID. No. 13 in native SA I/II. Thus, the nucleic acid sequences of the invention may be

extended at either or both of the 5' and 3' ends by any non-wild-type sequence.

The polypeptides of the invention are encoded by the DNA sequences described above. Thus, the polypeptides of the invention are not limited to the polypeptides of SEQ. ID. Nos. 1 to 11 although these sequences represent preferred polypeptides. Rather, the polypeptides of the invention also include polypeptides with sequences closely related to those of SEQ. ID. Nos. 1 to 11 that have one or more of the biological properties of SA I/II. These sequences may be prepared by altering those of SEQ ID Nos. 1 to 11 by any conventional method, or isolated from any organism or made synthetically. Such alterations, isolations or syntheses may be performed by any conventional method, for example by the methods of Sambrook et al (Molecular Cloning: A Laboratory Manual; 1989). In particular, polypeptides related to those of SEQ ID Nos. 1 to 11 may be prepared by modifying DNA sequences as shown in SEQ ID Nos. 12 to 22 expressing them recombinantly.

The polypeptides of the invention may be encoded by nucleic acid sequences that have less than 100% sequence identity with those of SEQ. ID. Nos. 12 to 22. Thus, polypeptides of the invention may include substitutions, deletions, or insertions, that distinguish them from SEQ. ID. Nos. 1 to 11 as long as these do not destroy the biological property or properties that the polypeptides have in common with SA I/II.

A substitution, deletion or insertion may suitably involve one or more amino acids, typically from one to five, one to ten or one to twenty amino acids, for example, a substitution, deletion or insertion of one, two, three, four, five, eight, ten, fifteen, or twenty amino acids. Typically, a polypeptide of the invention has at least 60%, at least 80%, at least 90%, or at least 95% sequence

identity to the sequence of any one of SEQ. ID. Nos. 1 to 11.

In general, the physicochemical nature of the sequence of SEQ. ID. Nos. 1 to 11 should be preserved in a polypeptide of the invention. Such sequences will generally be similar in charge, hydrophobicity and size to that of SEQ. ID. Nos. 1 to 11. Examples of substitutions that do not greatly affect the physicochemical nature of amino acid sequences are those in which an amino acid from one of the following groups is substituted by a different amino acid from the same group:

H, R and K
I, L, V and M
A, G, S and T
D, E, Q and N.

However, it may be desirable to alter the physicochemical nature of the sequence of SEQ. ID. Nos. 1 to 11 in order to increase its therapeutic effectiveness. For example, many of the amino acids in the polypeptides of the invention are acidic. For example, residues 975 to 1044 (SEQ. ID. No. 8) as a whole are of an acidic nature. This acidity is believed to facilitate binding to a mammalian tooth. Thus, it may be desirable to increase the acidity of polypeptides of the invention by adding acidic residues or by substituting acidic residues for non-acidic ones. Acidic residues include aspartic acid and glutamic acid.

Where polypeptides of the invention are synthesised chemically, D-amino acids (which do not occur in nature) may be incorporated into the amino acid sequence at sites where they do not affect the polypeptides biological properties. This reduces the polypeptides' susceptibility

to proteolysis by the recipient's proteases.

The nucleic acid sequences encoding the polypeptides of the invention may be extended at one or both ends by any non-wild-type sequence.

Thus, the polypeptides of the invention may be extended at either or both of the C- and N- termini by an amino acid sequence of any length. For example, an extension may comprise up to 5, up to 10, up to 20, up to 50, or up to 100 or 200 or more amino acids. An N-terminal extension may have any sequence apart from that which is N-terminal to the sequence of SEQ. ID. No. the invention (or the native sequence from which it is derived) in native SA I/II. A C-terminal extension may have any sequence apart from that which is C-terminal to the sequence of the invention (or the native sequence from which it is derived) in native SA I/II. Thus, the polypeptides of the invention may be extended at either or both of the C- and N- termini by any non-wild-type sequence.

The polypeptides of the invention may be attached to other polypeptides or proteins that enhance their antigenic properties. Thus, polypeptides of the invention may be attached to one or more other antigenic polypeptides. These additional antigenic polypeptides may be derived from S. mutans or from another organism. Possible additional antigenic polypeptides include heterologous T-cell epitopes derived from other S. mutans proteins or from species other than S. mutans. Heterologous B-cell epitopes may also be used. Such heterologous T-cell and or B-cell epitopes may be of any length and epitopes of up to 5, up to 10 or up to 20 amino acids in length are particularly preferred. These additional antigenic polypeptides may be attached to the polypeptides of the invention chemically. Alternatively, one or more additional antigenic sequences may comprise an extension to a polypeptide of the invention.

A polypeptide of the invention may be subjected to one or more chemical modifications, such as glycosylation, sulphation, COOH-amidation or acylation. In particular, polypeptides that are acetylated at the N-terminus are preferred, as are polypeptides having C-terminal amide groups. Preferred polypeptides may have one or more of these modifications. For example, particularly preferred peptides may have a C-terminal amide group and N-terminal acetylation.

A polypeptide of the invention may form part of a larger polypeptide comprising multiple copies of the sequence of one or more of SEQ. ID. Nos. 1 to 11 or a sequences related to them in any of the ways defined herein.

Polypeptides of the invention typically comprise at least 15 amino acids, for example 15 to 20, 20 to 50, 50 to 100 or 100 to 200 or 200 to 300 amino acids. Preferred polypeptides include those shown in SEQ. ID. Nos. 1 to 11.

Polypeptides according to the invention may be purified or substantially purified. Such a polypeptide in substantially purified form will generally form part of a preparation in which more than 90%, for example up to 95%, up to 98% or up to 99% of the peptide material in the preparation is that of a polypeptide or polypeptides according to the invention.

The nucleic acid sequences and polypeptides of the invention were originally derived from S. mutans. However, nucleic acid sequences and/or polypeptides of the invention may also be obtained from other organisms, typically bacteria, especially other streptococci. They may be obtained either by conventional cloning techniques or by probing genomic or cDNA libraries with nucleic acid sequences according to the invention. This can be done by any conventional method, such as the methods of Sambrook et

al (Molecular Cloning: A Laboratory Manual; 1989).

A nucleic acid sequence according to the invention may be included within a vector, suitably a replicable vector, for instance a replicable expression vector.

A replicable expression vector comprises an origin of replication so that the vector can be replicated in a host cell such as a bacterial host cell. A suitable vector will also typically comprise the following elements, usually in a 5' to 3' arrangement: a promoter for directing expression of the nucleic acid sequence and optionally a regulator of the promoter, a translational start codon and a nucleic acid sequence according to the invention encoding a polypeptide having one or more of the biological properties of SA I/II. A non-replicable vector lacks a suitable origin at replication whilst a non-expression vector lacks an effective promoter.

The vector may also contain one or more selectable marker genes, for example an ampicillin resistance gene for the identification of bacterial transformants. One particular preferred marker gene is the kanamycin resistance gene. Optionally, the vector may also comprise an enhancer for the promoter. If it is desired to express the nucleic acid sequence of the invention in a eucaryotic cell, the vector may also comprise a polyadenylation signal operably linked 3' to the nucleic acid encoding the functional protein. The vector may also comprise a transcriptional terminator 3' to the sequence encoding the polypeptide of the invention.

The vector may also comprise one or more non-coding sequences 3' to the sequence encoding the polypeptide of the invention. These may be from S. mutans (the organism from which the sequences of the invention are derived) or the host organism which is to be transformed with the vector or from another organism.

In an expression vector, the nucleic acid sequence of the invention is operably linked to a promoter capable of expressing the sequence. "Operably linked" refers to a juxtaposition wherein the promoter and the nucleic acid sequence encoding the polypeptide of the invention are in a relationship permitting the coding sequence to be expressed under the control of the promoter. Thus, there may be elements such as 5' non-coding sequence between the promoter and coding sequence. These elements may be native either to S. mutans or to the organism from which the promoter sequence is derived or to neither organism. Such sequences can be included in the vector if they enhance or do not impair the correct control of the coding sequence by the promoter.

The vector may be of any type. The vector may be in linear or circular form. For example, the vector may be a plasmid vector. Those of skill in the art will be able to prepare suitable vectors comprising nucleic acid sequences encoding polypeptides of the invention starting with widely available vectors which will be modified by genetic engineering techniques such as those described by Sambrook et al (Molecular Cloning: A Laboratory Manual; 1989). Preferred starting vectors include plasmids that confer kanamycin resistance and direct expression of the polypeptide of the invention via a tac promoter.

In an expression vector, any promoter capable of directing expression of a sequence of the invention in a host cell may be operably linked to the nucleic acid sequence of the invention. Suitable promoters include the tac promoter.

Such vectors may be used to transfect or transform a host cell. Depending on the type of vector, they may be used as cloning vectors to amplify DNA sequences according to the invention or to express this DNA in a host cell.

A further embodiment of the invention provides host cells harbouring vectors of the invention, i.e. cells transformed or transfected with vectors for the replication and/or expression of nucleic acid sequences according to the invention, including the sequences shown in SEQ. ID. Nos. 12 to 22. The cells will be chosen to be compatible with the vector and may for example be bacterial cells. Transformed or transfected bacterial cells, for example E.coli cells, will be particularly useful for amplifying nucleic acid sequences of the invention as well as for expressing them as polypeptides.

The cells may be transformed or transfected by any suitable method, such as the methods described by Sambrook et al (Molecular cloning: A Laboratory Manual; 1989). For example, vectors comprising nucleic acid sequences according to the invention may be packaged into infectious viral particles, such as retroviral particles. The constructs may also be introduced, for example, by electroporation, calcium phosphate precipitation, biolistic methods or by contacting naked nucleic acid vectors with the cells in solution.

In the said nucleic acid vectors with which the host cells are transformed or transfected, the nucleic may be DNA or RNA, preferably DNA.

The vectors with which the host cells are transformed or transfected may be of any suitable type. The vectors may be able to effect integration of nucleic acid sequences of the invention into the host cell genome or they may remain free in the cytoplasm. For example, the vector used for transformation may be an expression vector as defined herein.

The present invention also provides a process of producing polypeptides according to the invention. Such a process will typically comprise transforming or

transfecting host cells with vectors comprising nucleic acid sequences according to the invention and expressing the nucleic acid sequence in these cells. In this case, the nucleic acid sequence will be operably linked to a promoter capable of directing its expression in the host cell. Desirably, such a promoter will be a "strong" promoter capable of achieving high levels of expression in the host cell. It may be desirable to overexpress the polypeptide according to the invention in the host cell. Suitable host cells for this purpose include yeast cells and bacterial cells, for example E. coli cells, a particularly preferred E. coli strain being E. coli K12 strain BL 21. However, other expression systems can also be used, for example baculovirus systems in which the vector is a baculovirus having in its genome nucleic acid encoding a polypeptide of the invention and expression occurs when the baculovirus is allowed to infect insect cells.

The thus produced polypeptide of the invention may be recovered by any suitable method known in the art. Optionally, the thus recovered polypeptide may be purified by any suitable method, for example a method according to Sambrook et al (Molecular Cloning: A Laboratory Manual).

The polypeptides of the invention may also be synthesised chemically using standard techniques of peptide synthesis. For shorter polypeptides, chemical synthesis may be preferable to recombinant expression. In particular, peptides of up to 20 or up to 40 amino acid residues in length may desirably be synthesised chemically.

The nucleic acid sequences of the invention may be used to prepare probes and primers. These will be useful, for example, in the isolation of genes having sequences similar to that of SEQ. ID. No. 24. Such probes and primers may be of any suitable length, desirably from 10 to

100, for example from 10 to 20, 20 to 50 or 50 to 100 bases in length.

The present invention also provides antibodies to the polypeptides of the invention. These antibodies may be monoclonal or polyclonal. For the purposes of this invention, the term "antibody", includes fragments of whole antibodies which retain their binding activity for a target antigen. Such fragments include Fv, F(ab') and $F(ab')_2$ fragments, as well as single chain antibodies.

The antibodies may be produced by any method known in the art, such as the methods of Sambrook et al (Molecular Cloning: A Laboratory Manual; 1989). For example, they may be prepared by conventional hybridoma techniques or, in the case of modified antibodies or fragments, by recombinant DNA technology, for example by the expression in a suitable host vector of a DNA construct encoding the modified antibody or fragment operably linked to a promoter. Suitable host cells include bacterial (for example E. coli), yeast, insect and mammalian cells. Polyclonal antibodies may also be prepared by conventional means which comprise inoculating a host animal, for example a rat or a rabbit, with a peptide of the invention and recovering immune serum.

The present invention also provides pharmaceutical compositions comprising polypeptides of the invention. Three types of pharmaceutical compositions are particularly preferred. Firstly, compositions comprising polypeptides of the invention that include T-cell and/or B-cell epitopes may be used as vaccines against dental caries. Secondly, compositions comprising polypeptides of the invention that comprise adhesion sites will prevent or diminish adhesion of S. mutans to the tooth and can be used in the treatment of pre-existing cases of dental caries. Thirdly, compositions comprising polypeptides of the invention that

include both one or more antigenic (T-cell or B-cell) epitopes and one or more adhesion epitopes can be used to effect vaccination against dental caries at the same time as caring pre-existing cases of the disease. A similar effect can be achieved by including in a composition one or more peptides comprising one or more antigenic epitopes and one or more peptides comprising one or more adhesion sites.

A range of mammalian species can be vaccinated against dental caries using the polypeptides of the invention. Vaccination of humans is particularly desirable.

The compositions of the invention may be administered to mammals including humans by any route appropriate. Suitable routes include topical application in the mouth, oral delivery by means of tablets or capsule and parenteral delivery, including subcutaneous, intramuscular, intravenous and intradermal delivery. Preferred routes of administration are topical application in the mouth and injection, typically subcutaneous or intramuscular injection, with a view to effecting systemic immunisation.

As previously indicated, polypeptides according to the invention may also be mixed with other antigens of different immunogenicity.

The compositions of the invention may be administered to the subject alone or in a liposome or associated with other delivery molecules. The effective dosage depends on many factors, such as whether a delivery molecule is used, the route of delivery and the size of the mammal being vaccinated. Typical doses are from 0.1 to 100 mg of the polypeptide of the invention per dose, for example 0.1 to 1mg, and 1 to 5mg, 5 to 10mg and 10 to 100mg per dose. Doses of from 1 to 5 mg are preferred.

Dosage schedules will vary according to, for example, the route of administration, the species of the recipient

and the condition of the recipient. However, single doses and multiple doses spread over periods of days, weeks or months are envisaged. A regime for administering a vaccine composition of the invention to young human patients will conveniently be :6 months, 2 years, 5 years and 10 years, with the initial dose being accompanied by adjuvant and the subsequent doses being about ½ to ¼ the level of polypeptide in the initial dose. The frequency of administration can, however, be determined by monitoring the antibody levels in the patient.

Where the peptides of the invention are to be applied topically in the mouth, one preferred dosage regime is to apply one or more polypeptides of the invention on two or more occasions, for example 2 to 10 occasions over a period of a few weeks, for example one to six weeks. A particularly preferred regime of this type involves six applications of a polypeptide of the invention over a period of three weeks.

Typical doses for each topical application are in the range of 0.1 to 100mg for example 0.1 to 1mg, 1 to 10mg and 10 to 100mg. Doses of from 1 to 5mg for each application are preferred.

While it is possible for polypeptides of the invention to be administered alone it is preferable to present them as pharmaceutical formulations. The formulations of the present invention comprise at least one active ingredient, a polypeptide of the invention, together with one or more acceptable carriers thereof and optionally other therapeutic ingredients. The carrier or carriers must be "acceptable" in the sense of being compatible with the other ingredients of the formulation and not deleterious to the recipients thereof, for example, liposomes.

Formulations suitable for parenteral administration

include aqueous and non-aqueous sterile injection solutions which may contain anti-oxidants, buffers, bacteriostatis, bactericidal antibiotics and solutes which render the formulation isotonic with the blood of the intended recipient; and aqueous and non-aqueous sterile suspensions which may include suspending agents and thickening agents, and liposomes or other microparticulate systems which are designed to target the compound to blood components or one or more organs.

In particular, the polypeptides of the invention may be coupled to lipids or carbohydrates. This increases their ability to adhere to teeth, either by prolonging the duration of the adhesion or increasing its affinity, or both This is particularly desirable for shorter polypeptides of the invention, which comprise up to around 40 amino acid residues.

Of the possible formulations, sterile pyrogen-free aqueous and non-aqueous solutions are preferred. Also preferred are formulations in which the polypeptides of the invention are contained in liposomes. Injection solutions and suspensions may be prepared extemporaneously from sterile powders, granules and tablets of the kind previously described.

Oral methods of administration may produce an effect systemically or locally in the mouth. Orally active preparations can be formulated in any suitable carrier, such as a gel, toothpaste, mouthwash or chewing gum.

It should be understood that in addition to the ingredients particularly mentioned above the formulations of this invention may include other agents conventional in the art having regard to the type of formulation in question.

Accordingly, the present invention provides a method of vaccinating a mammalian host against dental caries or

treating dental caries, which method comprises administering to the host an effective amount of a pharmaceutical composition as described above, for example a vaccine composition.

Antibodies, including monoclonal antibodies, can be formulated for passive immunisation as indicated above for the formulation of including polypeptides of the invention. Preferred formulations for passive immunisation include solid or liquid formulations such as gels, toothpastes, mouth-washes or chewing gum.

A further aspect of the present invention is a naked nucleic acid vaccine. In this embodiment, the vaccine composition comprises a nucleic acid, typically an isolated nucleic acid, preferably DNA, rather than a polypeptide. The nucleic acid is injected in to a mammalian host and expressed in vivo, generating a polypeptide of the invention. This stimulates a T-cell response, which leads to protective immunity against dental caries in the same way as direct vaccination with a polypeptide of the invention.

Naked nucleic acid vaccination can be carried out with any nucleic acid according to the invention as long as it encodes a polypeptide that stimulates a T-cell and or B-cell response. Preferred nucleic acids are those shown in SEQ. ID Nos. 1 to 11. These will typically be included within an expression vectors as defined above. In such an expression vector, the nucleic acid according to the invention will typically be operably linked to a promoter capable of directing its expression in a mammalian host cell. For example, promoters from viral genes that are expressed in the mammalian cells such as the cytomegalovirus (CMV) immediate early gene promoter are suitable. Also suitable are promoters from mammalian genes that are expressed in many or all mammalian cell types such

as the promoters of "housekeeping" genes. One such promoter is the p-hydroxymethyl-CoA-reductase(HMG) promoter (Gautier et al (1989): Nucleic Acids Research; 17,8839).

For naked nucleic acid vaccination, it is preferred that the nucleic acid sequence according to the invention is incorporated into a plasmid vector, since it has been found that covalent closed circle (CCC) plasmid DNA can be taken up directly by muscle cells and expressed without being integrated into the cells' genomic DNA (Ascadi et al (1991): The New Biologist; 3, 71-81). Naked nucleic acid vaccine may be prepared as any of the types of formulation mentioned above in respect of conventional polypeptide-based vaccines. However, formulations suitable for parenteral injection, especially intramuscular injection, are preferred. Naked nucleic acid vaccines may be delivered in any of the ways mentioned above in respect of conventional polypeptide-based vaccines but intramuscular injection is preferred.

Accordingly, the present invention provides a vaccine composition comprising a nucleic acid sequence or vector as described above and an acceptable carrier.

The following examples illustrate the invention.

EXAMPLES

MATERIALS AND METHODS

Materials. Fmoc amino acids, benzotriazole-1-yl-oxy-tris-pyrrolidino-phosphonium hexaflurophosphate (PyBOP) and Rink Amide MBHA resin were purchased from Calbiochem-Novabiochem (UK) Ltd., (Nottingham, UK). Dimethylformamide, trifluoroacetic acid, diethyl ether, dichloromethane and piperidene were purchased from Romil Chemicals Ltd (Loughborough, UK). Di-isopropylethylamine was from Aldrich Chemical Co. (Dorset, UK). Oligonucleotides were purchased from Oswel DNA Service (University of Edinburgh,

Edinburgh, UK).

Bacteria and growth conditions

S.mutans Guy's strain (serotype c) were grown in 10L basal medium supplemented as described previously (Russel et al (1978): Arch. Oral Biol., 2317; Russel et al (1980): Infect, Immun. 61, 5490) at 37°C for 72h for SA I/II preparation. For the adhesion assay, S. mutans were grown in Todd-Hewitt broth (Difco Laboratories, Detroit, Mich.). Escherichia coli BL21 (DE3) (Novagen Inc., Madison, Wis.) harbouring pET15b were grown at 37°C in Luria-Bertani broth supplemented with carbenicillin (50μg/ml) and recombinant protein expression was induced with isoppropyl-6-D-thiogalactopryanoside (1mM).

Antigens. SA I/II was prepared from S. mutans (serotype c, Guy's strain) as described by Russel et al (1980: Infect. Immun. 28, 486). Using the procedure of Munro et al (1993: Infect. Immun. 61, 4590), the portion of the gene encoding residues 984-1161 was amplified by using the oligonucleotide primers: (5') ATACATATGCCAACTGTTCATTTCCATTACTTT (SEQ. ID. No. 25) and (3') GCCATTGTCGACTCATTCATTTTTATTAACCTTAGT (SEQ. ID. No. 26), cloned into pET15b (modified by the addition of a Sal I site) and expressed in E. coli.

Synthetic peptides. Peptide amides (20 mers overlapping by 10 residues) were synthesised on Rink amide MBHA resin in sealed porous polypropylene bags by the manual simultaneous multiple peptide synthesis procedure (Houghten (1985) PNAS 892, 5131) using Fmoc chemistry. PyBOP was used as coupling agent and Fmoc amino acids were activated in situ by addition of diisopropylethylamine. Following 20 cycles of synthesis, resin was washed with dimethylformamide followed by dichloromethane and peptides were cleaved by

incubation in trifluoroacetic acid- ethanedithiol-anisole-phenol-H_2O (82.5:2.5:5:5:5; v/v/v/w/v) for 2h at room temperature. Peptides were precipitated by the addition of 5 volumes ether, recovered by centrifugation and washed three times with ether. Finally, peptides were dissolved in water and lyophilised. The scale of synthesis was 50μmol. Aliquots of each peptide were hydrolysed in 6M HCl at 110°C for 24h and compositions were determined using the Beckman 121MB automated analyser (Beckman Instruments Ltd, Bucks, UK). In each case the composition matched that predicted.

Antibodies. MAbs, L243 (anti-MHC class II) and W6/32 (anti-MHC class I) were produced from cultures of hybridomas obtained from the American Type Culture Collection (Rockville, Md, USA). ID4 an isotype (IgG2a) matched control of irrelevant specificity was provided by Dr. P. Shepherd (Department of Immunology, UMDS, Guys Hospital, London, UK). Rabbit anti-SA I/II antiserum was prepared as described previously (Russel et al (1980). Infect. Immun. 28, 486).

Lymphoproliferative assay. Defibrinated blood from volunteers was separated on a Ficoll gradient. Sera was used for antibody assays (see below) while peripheral blood mononuclear cells (PBMCs) were washed and resuspended in RPMI 1640 (Sigma Chemical Co., St. Louis, Mo, USA) supplemented with 2mM L-glutamine, penicillin (100 IU/ml), streptomycin sulphate (100 μg/ml) and 10% heat-inactivated autologous serum. PBMCs (10^5 cells/well) were cultured in 96-well round-bottomed plates (Costar, Cambridge, Mo, USA) in a total volume of 200 μl. Three replicates of each culture were incubated with three concentrations (1, 10 and 40 μg/ml) of SA I/II, recombinant fragments, non-

recombinant control or synthetic peptides. Incubation was at 37°C in a humidified atmosphere with 5% CO_2 for 6 days. Each culture received 0.2 μCi (7.4kBq) of [^{3}H]-thymidine (Amersham International, Bucks, UK) 6h before harvesting. Cultures were harvested onto glass fibre filters using a Dynatech (Chantilly, VA, USA) Minimal Cell harvester and [^{3}H]-thymidine incorporation was measured using the LKB liquid scintillation counter (Bromma, Sweden). Proliferation was expressed as stimulation index which is mean counts per minute (cpm) of antigen-stimulated, divided by cpm of antigen-free cultures. Concanavalin A (10 μg/ml) (Sigma Chemical Co., St. Louis, Mo, USA) was used with every culture as a positive control but the results are not presented.

MHC dependency of proliferative responses to SA I/II was determined by culturing cells with antigen (10 μg/ml) as above in the presence of MAbs L235, W6/32 or ID4 at 1, 10 and 20 μg/ml. Cultures were incubated with [^{3}H]-thymidine, harvested and [^{3}H]-thymidine uptake was determined as described above.

ELISA for serum antibodies. Antibody recognition of synthetic peptides was determined by ELISA. Peptides (10 μg/ml) in phosphate buffered saline (PBS) were adsorbed to wells of polystyrene microtitre plates (Dynatech) for 2h at room temperature. Plates were washed and wells were treated with 1.5% (w/v) bovine serum albumen (BSA) for 1h at room temperature to block unbound sites. After washing, bound peptides were incubated with serially diluted sera in duplicate. Bound IgG antibodies were determined by incubation with alkaline phosphate conjugated-goat anti-human Ig (Sigma Chemical Co.) and subsequent reaction with paranitrophenyl phosphate (Sigma Chemical Co.). Plates were read at 405nm using the microplate reader model 450

(Bio-Rad). After initial screening, the assay was repeated at least 3 times with each serum using a restricted set of peptides. SA I/II (2 μg/ml) was included in each assay as was an irrelevant peptide (HQAAMQIIRDIINEEAADWD(SEQ. ID. No. 27) derived from the sequence of SIV p27. Results are expressed as the highest dilution giving an absorbance ≥0.2.

Western blotting. Serum antibody responses were also assayed by Western blotting using SA I/II, the recombinant polypeptides and a control fraction from E. coli BL21 harbouring non-recombinant pET15b. Purified antigens were separated by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) with gels of 10% acrylamide, by using a mini-gel system (Hoeffer Scientific Instruments, San Francisco, Co, USA). Proteins were transferred to nitrocellulose with a semi-dry blotter (Sartorius A.G., Gottingen, Germany). Nitrocellulose strips were blocked with 5% (wt/vol) nonfat milk powder 2.5% (wt/vol) BSA in Tris-HCl-buffered saline (pH 8.0) containing 0.05% (wt/vol) Tween 20. Strips were subsequently incubated with human sera (1 in 20 dilution) or rabbit anti-SA I/II antiserum (10^{-4} dilution) and bound antibody was visualised by using alkaline phosphatase-conjugated secondary antibody with 5-bromo-4-chloro-3- indolylphosphate and nitroblue tetrazolium (Sigma Chemical Co.) as substrates. Each sera was assayed three times and responses were considered as positive if bands were visible in at least two assays.

Bacterial adherence assay. SA I/II mediated adherence of S. mutans (Guy's strain) to saliva was assayed by determining binding of [^{3}H]-thymidine labelled bacteria to saliva adsorbed to microtitre wells. Freshly collected human saliva from a single donor was clarified by

centrifugation for 10 min at 3000g, heat-inactivated at 60°C for 30 min and finally clarified by centrifugation at 17,000g for 20 min. Treated saliva was diluted with an equal volume of PBS and adsorbed to the wells of a polystyrene 96-well flat-bottomed microtitre plate (Immulon 4; Dynatech) for 2h at room temperature. After coating, wells were washed three times with PBS and unbound sites were blocked by incubation with 1.5% (wt/vol) BSA in PBS for 1h at room temperature. Plates were then washed three times with 50 mM KCl-1mM $CaCl_2$-38 mM $MgCl_2$-1 mM KH_2PO_4-1.2 mM K_2PO_4 (pH 7.2; adherence buffer). S. mutans cells from an overnight culture in Todd-Hewitt broth were used to inoculate (1/10 volume) a further culture in Todd-Hewitt broth containing 100μCi (3.7 MBq) [^{3}H]-thymidine (Amersham International plc) per ml. Cells were harvested in late log phase (O.D. 700nm approximately 0.4) pelleted by centrifugation at 1000g for 10 min and washed three times in adherence buffer. The final suspension was vortexed with 0.5 volume glass beads to break up chains of cocci which was monitored microscopically (Munro et al (1993): Infect. Immun. 61, 4590). Cells were resuspended to $5x10^4$ c.p.m. per 50 μl and BSA was added to 1.5% (wt/vol). Specific activity of the washed S. mutans cells was estimated to be $1.3x10^{-3}$ c.p.m. per cell (Munro et al (1985): Infect. Immun. 61, 4590). In competitive inhibition of adherence, the various synthetic peptides were added to the wells (at final concentrations 62.5-500μM) in 50 μl adherence buffer containing 1.5% (wt/vol) BSA together with 50 μl radiolabelled S. mutans suspension. Microtitre plates were incubated at 37°C for 2h with gentle shaking and subsequently were washed ten times with adherence buffer. Bound S. mutans cells were eluted with 1% (wt/vol) SDS and transferred to glass fibre filters by using the Micromate 196 cell harvester (Canberra Packard,

Berks, UK). Filters were counted using the Matrix 96 direct beta counter (Canberra Packard). Background binding was determined on wells to which no saliva was adsorbed. The percentage of binding of S. mutans to saliva was calculated by the formula [(test c.p.m.) - (control c.p.m.) / total c.p.m.] x100. Percent inhibition of adherence was calculated as [(percent adherence without inhibitor-percent adherence with inhibitor) / percent adherence without inhibitor] x100. For proteins, determinations of streptococcal adhesion were made in triplicate or quadruplicate at each protein concentration while for peptides, duplicate determinations were made. In each case the assay was performed at least three times.

Statistics

The student's t test was used to analyse results.

Example 1

Preparation of a panel of overlapping synthetic peptides and analysis of their properties.

T cell epitope mapping

A panel of 32 overlapping synthetic peptides, spanning residues 803-1174 of SA I/II, was prepared, as described above (See Figure 1). Proliferative responses of PBMCs from 30 subjects were determined by stimulation with peptides (see Figure 2). All subjects responded to at least one peptide with a band range of 1-8 peptides, and a mean of 4.4 peptides. On the basis of frequency of response to each peptide ($SI \geq 3.0$ c.p.m. >500) 3 immunodominant epitopes were identified; peptides 803-822, 975-994 and 985-1004, each yielding frequencies >50% (Fig. 1). Since most (13/15) subjects who responded to peptide 975-994 also responded to peptide 985-1004, it is probable

that a single T-cell epitope is present within residues 975-1004. Minor T cell epitopes were also identified within peptides 1005-1024, 1015-1034, 1085-1104 and 1115-1134 with frequencies >20% and some of the adjacent peptides may represent single T cell epitopes.

MHC restriction of the lymphoproliferative responses (See Figure 3 and Table 2)

HLA restriction of the T cell response was first studied by dose-dependant inhibition with MAb to HLA class I and II antigen (Fig. 2). The lymphoproliferative response was inhibited by 50% with 1μg of MAb to HLA class II (L243) and 10μg of the MAb inhibited 100% of the responses (from SI 10.0$\pm$3.2 to SI 1.5$\pm$0.4). Neither MAb to HLA class I (W6/32) nor the isotype control induced any inhibition of the lymphoproliferative response.

The HLA-DR of 17 subjects were determined and 6 of these were homozygous. The responses of the immunodominant and minor epitopes were then studied in the 6 DR homologous subjects (Table 2). Only peptide 975-994 appeared to be restricted by HLA-DR1. The other 6 peptides stimulated lymphocytes from HLA-DR1, 2 (except AA 1085-1104) and DR6 (except AA 803-822). DR5 was restricted by peptide 803-922, though the latter stimulated lymphocytes with DR1, 2 and 3 antigens. Lymphocytes with DR3 or 4 antigen responded to 3 or 4 peptides. The results suggest that except for peptide 975-994, the remaining 6 peptides appear to be promiscuous as they stimulated lymphocytes with 3 to 5 HLA-DR antigens.

Table 1

DR	803-822	975-994	985-1004	1005-1024	1014-1034	1085-1104	1115-1134
1	**4.1±1.0**	**4.0±1.3**	**5.8±1.8**	**3.2±0.6**	**3.3±1.1**	**3.3±1.3**	**3.2±0.6**
2	**19.3±6.6**	2.2±0.4	**16.7±1.7**	**14.6±5.7**	**11.2±5.2**	0.6±0.3	**14.7±3.3**
3	**6.1±2.7**	0.7±0.2	**4.1±2.3**	1.0±0.2	2.1±1.7	**4.3±1.2**	1.9±2.3
4	2.5±0.8	1.8±0.7	**3.0±0.3**	**3.2±0.5**	1.6±0.1	**3.7±0.6**	1.5±0.7
5	**6.8±1.0**	1.8±1.3	2.0±0.8	2.3±0.5	1.3±0.3	1.2±0.4	2.9±2.8
6	2.6±1.5	2.9±0.9	**3.5±0.5**	**8.3±3.1**	**5.7±2.4**	**5.6±1.4**	**5.0±2.0**

Table 1 The relationship between HLA-DR1-6 and the T cell responses to 7 synthetic peptides.
S.I (±sem) values of subjects homozygous for DR are shown.
Positive responses (S.I.>3.0,c.p.m.>500) are in bold.

B cell epitope mapping (see Figure 4)

Recognition of the recombinant fragments was assessed by Western blotting. Representative blots obtained with sera from 3 individuals are shown in Fig. 3 together with a positive control using rabbit anti-SA I/II antiserum. In panel a, SA I/II, and 984-1161 were recognised strongly. Rabbit anti-SA I/II antiserum used as a positive control (panel d) recognised recombinant 984-1161. The recombinant polypeptide corresponding to residues 984-1161 was also analysed. SA I/II was recognised by all subjects. B cell epitopes were mapped by ELISA using the panel of synthetic peptides. The panel of peptides was screened with sera from 22 individuals and 8 peptides which were recognised by more than one individual, together with one peptide which was not recognised, were selected for further analyses (Fig.5). SA I/II was recognised by all subjects with mean $\log_2$ titre of 7.6 ± 1.2. Titres against peptides were lower, with only that against peptide 824-843 (mean $\log_2$ titre 4.7 ± 1.1) being significantly greater than the titre against the control SIV p27 peptide ($t=7.28$ $p<0.01$). The proportion of significant titres (>mean +2 standard derivations) was also calculated (Fig. 5) and only peptide 824-843 showed high frequency (18/22). Indeed, an immunodominant B cell epitope is present within peptide 824-843, possibly shared with the overlapping peptide 834-853, while peptides 925-944, 1035-1054 and 1085-1104 constitute minor B cell epitopes. Despite the high frequency of responses to the recombinant polypeptide 984-1161 described above), a very low frequency of responses was observed to peptides within this region.

Saliva samples from the subjects were cultured to determine levels of S. mutans. In 66% of individuals S. mutans was detected (range 10^3-10^5 colony forming units/ml). There was no correlation between S. mutans

levels and recognition of particular epitopes or titre against SA I/II.

Adhesion epitope mapping

Adherence of S. mutans to saliva-coated microtitre wells (a model of the tooth surface) was determined with [^{3}H]-thymidine labelled S. mutans. The proportion of adhering bacteria was in the range 1-5%. In the absence of saliva, the proportion of adhering bacteria was <0.1%.

In a series of competitive inhibition assays, the panel of synthetic peptides was assayed for inhibition of adhesion of S. mutans to saliva-coated microtitre wells. Peptides 1005-1024, 1025-1044 and 1085-1104 consistently inhibited adhesion with maximal inhibition $\geq$ 90% at concentrations of 500 μM (Fig. 6). Adjacent peptides 1015-1034 and 1095-1114 showed more variable and lower inhibition, and may be part of the adhesion epitopes.

Example 2

Construction of an expression vector and expression of a recombinant polypeptide of the invention (SEQ. ID. No. 8). Using the oligonucleotide primers TAT CAT ATG CAA GAT CTT CCA ACA CCT CCA TCT ATA (5') (SEQ. ID. NO. 29) and GTC GAC TCA TAC CAA GAC AAA GGA AGT TGT (3') (SEQ. ID. No.30) the portion of the SA I/II gene encoding residues 975-1044 (SEQ. ID. No.8) was amplified by polymerase chain reaction. The amplified gene fragment (with introduced Nde I and Sal I restriction enzyme sites) was cloned using the Ta cloning system and was subcloned into the plasmid pET15b. The recombinant polypeptide was expressed in E. coli BL21 (DE3).

Example 3

Stimulation of an in vitro T-cell response by the

recombinant polypeptide (SEQ. ID. No. 8).

Peripheral blood lymphocytes from human volunteers were prepared as described above. Cells were incubated with purified recombinant polypeptide 975-1044 at concentrations of 40, 10 and 1μg/ml. Cells were also incuabted with a protein fraction prepared in the same way from E. coli harbouring non-recombinant plasmid. Proliferative responses of 17 subjects were determined. Mean stimulation index (±sem) was 11.6±2.3 compared with 2.4±0.3 for the control. The frequency of subjects responding (i.e. those with stimulation index ≥ control + 2SD) was 15/17.

Example 4

Immunisation of mice with the recombinant polypeptide (SEQ. ID. NO. 8) (See Figure 7)

i) Groups of mice (3-4 per group) were immunised with 975-1044 (SEQ. ID. No. 8) by two routes:

a) intraperitoneally with 50μg polypeptide in incomplete Freund's adjuvant with a boost after 4 weeks (also 50μg in incomplete Freund's adjuvant and intraperitoneally).

b) subcutaneously. A single immunisation with 50μg polypeptide in incomplete Freund's adjuvant.

ii) Draining lymph nodes were removed 10 to 14 days after immunisation, pooled and homogenised to give a single cell suspension in RPMI 1640 culture medium supplemented with 2mM glutamine, 1mM pyruvate, 50mM 2-mercaptoethanol, 100u/ml penicillin, 100μg/ml streptomycin, 100mM HEPES and 5% foetal calf serum. Cells ($2x10^5$/well) were cultured with antigen and proliferation was measured by incorporation of [^{3}H]-thymidine as described above. Antigens were SA I/II recombinant polypeptides, peptides spanning residues 975-1044 and a control protein fraction from E. coli harbouring non-recombinant plasmid.

As in Fig 7, all mouse strains responded to SA I/II

and the recombinant polypeptide 975-1044 (SEQ. ID. No. 8). Positive responses to peptides were those of stimulation index $\geq$3.0 (cpm>500). SJL mice responded to peptide 985-1004 and DBA/a mice responded to peptide 975-995 and 985-1004. For BALB/c mice, no significant responses to peptides were observed although the response to peptide 985-1004 was greater than responses to the remaining peptides.

iii) **Antibody recognition (See Table 2)**

Sera from mice immunised intraperitoneally with polypeptide 975-1044 recognised intact cells of S.mutans, intact SA I/II and recombinant 975-1044. Peptides 995-1014 and 1025-1044 were also recognised. The titre for each strain was as in Table 2, which shows $\log_2$ titres where initial dilution was 1 in 50 (titre = 1).

Table 2

Antibody recognition of S.mutans, SA I/II and peptides.

STRAIN	ANTIGEN									
	S.mutans	SA I/II	975-1044	NR Control	PEPTIDES					
					975-994	985-1004	995-1014	995-1014	1015-1034	1025-1044
SJL	4.0	4.0	10.3	-	-	-	8.7	1.0	-	5.7
DBA/1	3.0	2.7	10.7	-	0.7	0.7	4.7	2.0	-	7.3
BALB/C	2.0	2.8	10.7	-	-	-	5.7	3.0	-	4.7

Numbers in the table are $\log_2$ titres (1=1:50)

Example 5

Analysis of the interaction between streptococcal antigen I/II and salivary receptor using BIAcore

AIMS

In this study, we have used surface plasmon resonance (spr) to analyse the interaction between purified SA I/II and whole human saliva or purified salivary receptor. In addition we have investigated the calcium dependence of binding, identified individual amino acid residues which may be involved in binding and determined the affinity of the interaction between SA I/II and salivary receptor.

METHODS

Materials

SA I/II and recombinant polypeptides were prepared as described above. Salivary receptor was prepared by absorption of whole saliva with intact cells of *S. mutans* (Lee et al (1989) Infect. Immun. 57:3306-3313). The cells were washed with KPBS (2.7 mM KCl, 137mM NaCl in 1.5mM KH_2PO_4, 6.5mM Na_2HPO_4, pH 7.2)and adsorbed material was eluted with 1mM EDTA in KPBS. Analysis of the purified material by polyacrylamide gel electrophoresis in the presence of Na dodecyl sulphate indicated the presence of components of Mr>200,000 and approximately 40,000. Peptides were prepared by the simultaneous multiple peptide synthesis procedure (Houghten (1985) Proc. Natl. Acad. Sci. USA 82: 5131-5135) as above. In addition, a series of peptides was synthesised corresponding to residues 1025-1044 in which each residue in turn was substituted by alanine.

Binding Analyses

Purified SA I/II or salivary receptor was immobilised on the sensor chip surface at a concentration of 100 μg/ml in 10mM Na formate pH 3.5 using the amine coupling kit (Pharmacia Biosensor).

i. Inhibition studies

Binding of immobilised SA I/II to receptors in whole saliva was determined in the absence and presence of inhibitors (at varying concentrations). Inhibition by alanine-substituted peptides was analysed at a peptide concentration of 50 μM. The running buffer was HEPES buffered saline (HBS) and the surface was regenerated with 100mM HCl.

ii. Direct binding

Purified salivary receptor was immobilised on the sensor chip and binding of SA I/II or purified recombinant polypeptide fragments was determined.

RESULTS

i. Calcium dependency

In separate determinations with whole saliva, binding to immobilised SA I/II varied from approximately 250 resonance units (RU) - 800 RU. In the presence of EDTA, binding was inhibited with maximal inhibition of 95% at a concentration of 10mM EDTA. Subsequent binding assays were performed in the presence of 5mM calcium.

ii. Inhibition of binding

Purified SA I/II or recombinant polypeptide fragments 1 (residues 39-481), 2 (residues 475-824), 3 (residues 816-1213), 4 (residues 1155- 1538) and recombinant 984-1161 were added to fluid phase saliva as competitive inhibitors at concentrations varying from 0-20μM. SA I/II inhibited binding most efficiently with approximately 90% inhibition

at a concentration of 6 μM (Fig.8). Of the recombinant fragments, only fragment 3 and r984-1161 inhibited binding to salivary receptors to a significantly greater extent than the control (bovine serum albumin) with maximal inhibition of 65% and 50%, respectively (Fig. 8).

A panel of synthetic peptides (20mers overlapping by 10) spanning residues 803-1174 was assayed for inhibitory activity. Peptide 1025-1044 was the most effective inhibitor although 10-20 fold higher concentrations were required than for polypeptides (Fig.9). A panel of peptides in which each of the residues 1025-1044 in turn were substituted with alanine (alanine was substituted by serine where it occurred naturally) was also analysed for inhibitory activity. Substitution of Glu (1037) consistently abolished inhibition mediated by the peptide (Fig.10). Similarly, substition of Gln 1025, Thr 1039, Phe 1041, Val 1042, Leu 1043 and Val 1044 reduced the inhibition of binding which was mediated by the peptide 1025-1044.

iii. Direct Binding

For these analyses, purified salivary receptor was immobilised on the sensor chip and binding to fluid phase SA I/II or recombinant polypeptides was determined. At a concentration of approximately 5 μM both SA I/II and recombinant SA I/II bound to salivary receptor in the range 500-600 RU (Fig.11). Binding of recombinant polypeptides was determined at a concentration of approximately 20μM and highest binding was obtained with fragment 3 (1256 RU) (Fig.11). Binding of other fragments although significantly greater than the myosin control was not greater than the bovine serum albumin control and thus does not appear to be specific. Addition of EDTA (10 μM) in

this assay completely inhibited binding of fluid phase SA I/II.

Affinity and rate constants for the adhesin-receptor interaction were determined for SA I/II, recombinant SA I/II and fragment 3 (Table 3). The values indicate a low affinity interaction with a slow association rate constant and a relatively rapid dissociation constant.

Conclusions

These analyses confirm that residues 816-1213 of SA I/II form an adhesion binding region and that within this region, peptide 1025-1044 forms an adhesion epitope. We have now extended these findings by identifying specific residues which may be essential for binding to salivary receptor, namely residues 1025, 1037, 1039 and 1041-1045. The binding is EDTA sensitive and, under the assay conditions, is of relatively low affinity.

TABLE 3

		SA I/II	recomb.SA I/II	FRAG 3
k_a	(M^{-1} s^{-1})	n.d.	20.9×10^3	1.5×10^3
k_d	(s^{-1})	2×10^{-2}	4.2×10^{-3}	8.1×10^{-3}
K_A	(M^{-1})	n.d.	5.0×10^6	0.2×10^6

n.d. not determined

SEQUENCE INFORMATION

As a result of the experiments detailed above, the following sequences have been identified as being of particular interest.

(i) **Residues 925 to 1114 (SEQ. ID. No. 1).** This sequence comprises sequences (iv) and (v) below and includes 2 series of overlapping T-cell, B-cell and adhesion epitopes, a further B-cell epitope, a further T-cell epitope and an adhesion site.

SEQ. ID. No. 1:

TEKPLEPAPVEPSYEAEPTPPTPTPDQPEPNKPVEPTYEVIPTPPTDPVYQDLPTPPSI
PTVHFHYFKLAVQPQVNKEIRNNNDVNIDRTLVAKQSVVKFQLKTADLPAGRDETTSFV
LVDPLPSGYQFNPEATKAASPGFDVAYDNATNTVTFKATAATLATFNADLTKSVATIYP
TVVGQVLNDGATY

Its DNA sequence is (SEQ.ID.No. 12):

ACAGAAAAGCCGTTGGAGCCAGCACCTGTTGAGCCAAGCTATGAAGCAGAGCCAACGCCA
CCGACACCAACACCAGATCAACCAGAACCAAACAAACCTGTTGAGCCAACTTATGAGGTT
ATTCCAACACCGCCGACTGATCCTGTTTATCAAGATCTTCCAACACCTCCATCTATACCA
ACTGTTCATTTCCATTACTTTAAACTAGCTGTTCAGCCGCAGGTTAACAAAGAAATTAGA
AACAATAACGATGTTAATATTGACAGAACTTTGGTGGCTAAACAATCTGTTGTTAAGTTC
CAGCTGAAGACAGCAGATCTCCCTGCTGGACGTGATGAAACAACTTCCTTTGTCTTGGTA
GATCCCCTGCCATCTGGTTATCAATTTAATCCTGAAGCTACAAAAGCTGCCAGCCCTGGC
TTTGATGTCGCTTATGATAATGCAACTAATACAGTCACCTTCAAGGCAACTGCAGCAACT
TTGGCTACGTTTAATGCTGATTTGACTAAGTCAGTGGCAACGATTTATCCAACAGTGGTC
GGACAAGTTCTTAATGATGGCGCAACTTAT

(ii) **Residues 1005 to 1044 (SEQ. ID. No. 2).** This comprises

a T-cell epitope overlapping two adhesion sites.

SEQ. ID. No. 2

NNNDVNIDRTLVAKQSVVKFQLKTADLPAGRDETTSFVLV

Its DNA sequence is (SEQ. ID. No. 13):

AACAATAACGATGTTAATATTGACAGAACTTTGGTGGCTAAACAATCTGTTGTTAAGTTC
CAGCTGAAGACAGCAGATCTCCCTGCTGGACGTGATGAAACAACTTCCTTTGTCTTGGTA

(iii) Residues 1085-1104 (SEQ. ID. No. 3). Here, a T-cell epitope, a B-cell epitope and an adhesion site overlap.

SEQ. ID. No. 3:

LATFNADLTKSVATIYPTVV

Its DNA sequence is (SEQ. ID. No. 14):

TTGGCTACGTTTAATGCTGATTTGACTAAGTCAGTGGCAACGATTTATCCAACAGTGGTC

(iv) Residues 1005 to 1114 (SEQ. ID. No. 4). This comprises sequences (ii) and (iii) above and therefore includes two sequences in which a B-cell epitope a T-cell epitopes and an adhesion site overlap.

SEQ. ID. No. 4

NNNDVNIDRTLVAKQSVVKFQLKTADLPAGRDETTSFVLVDPLPSGYQFNPEATKAASPGF
DVAYDNATNTVTFKATAATLATFNADLTKSVATIYPTVVGQVLNDGATY

Its DNA sequence is (SEQ. ID. No. 15):

AACAATAACGATGTTAATATTGACAGAACTTTGGTGGCTAAACAATCTGTTGTTAAGTTC
CAGCTGAAGACAGCAGATCTCCCTGCTGGACGTGATGAAACAACTTCCTTTGTCTTGGTA

GATCCCCTGCCATCTGGTTATCAATTTAATCCTGAAGCTACAAAAGCTGCCAGCCCTGGC
TTTGATGTCGCTTATGATAATGCAACTAATACAGTCACCTTCAAGGCAACTGCAGCAACT
TTGGCTACGTTTAATGCTGATTTGACTAAGTCAGTGGCAACGATTTATCCAACAGTGGTC
GGACAAGTTCTTAATGATGGCGCAACTTAT

(v) **Residues 925 to 1004 (SEQ. ID. No. 5).** This comprises a B-cell epitope, an immunodominant T-cell epitope and an adhesion site.

SEQ. ID. No. 5:

TEKPLEPAPVEPSYEAEPTPPTPTPDQPEPNKPVEPTYEVIPTPPTDPVYQDLPTPPSIPT
VHFHYFKLAVQPQVNKEIR

Its DNA sequence is (SEQ. ID. No. 16):

ACAGAAAAGCCGTTGGAGCCAGCACCTGTTGAGCCAAGCTATGAAGCAGAGCCAACGCCA
CCGACACCAACACCAGATCAACCAGAACCAAACAAACCTGTTGAGCCAACTTATGAGGTT
ATTCCAACACCGCCGACTGATCCTGTTTATCAAGATCTTCCAACACCTCCATCTATACCA
ACTGTTCATTTCCATTACTTTAAACTAGCTGTTCAGCCGCAGGTTAACAAAGAAATTAGA

(vi) **Residues 925 to 1054 (SEQ. ID. No. 6).** This comprises sequence (v) above, together with a further adjacent adhesion site and a further overlapping B-cell epitope.

SEQ ID. No. 6

TEKPLEPAPVEPSYEAEPTPPTPTPDQPEPNKPVEPTYEVIPTPPTDPVYQDLPTPPSIPT
VHFHYFKLAVQPQVNKEIRNNNDVNIDRTLVAKQSVVKFQLKTADLPAGRDETTSFVLVDP
LPSGYQFN

Its DNA sequence is (SEQ. ID. No. 17):

ACAGAAAAGCCGTTGGAGCCAGCACCTGTTGAGCCAAGCTATGAAGCAGAGCCAACGCCA
CCGACACCAACACCAGATCAACCAGAACCAAACAAACCTGTTGAGCCAACTTATGAGGTT
ATTCCAACACCGCCGACTGATCCTGTTTATCAAGATCTTCCAACACCTCCATCTATACCA
ACTGTTCATTTCCATTACTTTAAACTAGCTGTTCAGCCGCAGGTTAACAAAGAAATTAGA
AACAATAACGATGTTAATATTGACAGAACTTTGGTGGCTAAACAATCTGTTGTTAAGTTC
CAGCTGAAGACAGCAGATCTCCCTGCTGGACGTGATGAAACAACTTCCTTTGTCTTGGTA

GATCCCCTGCCATCTGGTTATCAATTTAAT

(vii) **Residues 803-854 (SEQ. ID. No. 7).** This comprises a major T-cell epitope and adjacent immunodominant B-cell epitope.

SEQ. ID. No. 7:

ETGKKPNIWYSLNGKIRAVNLPKVTKEKPTPPVKPTAPTKPTYETEKPLKPA

Its DNA sequence is (SEQ. ID. No. 18)

GAAACCGGCAAAAAACCAAATATTTGGTATTCATTAAATGGTAAAATCCGTGCGGTTAAT
CTTCCTAAAGTTACTAAGGAAAAACCCACACCTCCGGTTAAACCAACAGCTCCAACTAAA
CCAACTTATGAAACAGAAAAGCCATTAAAACCGGCA

(viii) **Residues 975 to 1044 (SEQ. ID. No. 8).** This comprises a T-cell epitope, a B-cell epitope and an adhesion site.

SEQ. ID No. 8:

QDLPTPPSIPTVHFHYFKLAVQPQVNKEIRNNNDVNIDRTLVAKQSVVKFQLKTADLPAGR
DETTSFVLV

Its DNA sequence is (SEQ. ID. No. 19):

CAAGATCTTCCAACACCTCCATCTATACCAACTGTTCATTTCCATTACTTTAAACTAGCT
GTTCAGCCGCAGGTTAACAAAGAAATTAGAAACAATAACGATGTTAATATTGACAGAACT
TTGGTGGCTAAACAATCTGTTGTTAAGTTCCAGCTGAAGACAGCAGATCTCCCTGCTGGA
CGTGATGAAACAACTTCCTTTGTCTTGGTA

(ix) **Residues 1024 to 1044 (SEQ. ID. No. 9).** This comprises a T-cell epitope overlapping with an adhesion site.

SEQ. ID. No. 9
FQLKTADLPAGRDETTSFVLV

Its DNA Sequence is (SEQ. ID. No. 20):

TTCCAGCTGAAGACAGCAGATCTCCCTGCTGGACGTGATGAAACAACTTCCTTTGTCTTG
GTA

(x) **Residues 803 to 1114 (SEQ. ID. No. 10).** This comprises sequences (i) and (vii) above and some intervening sequence. Residues 803 to 1114 comprise 2 series of overlapping T-cell, B-cell and adhesion epitopes, a further T-cell epitope and a further adhesion site and an immunodominant B-cell epitope and a major T-cell epitope.

SEQ. ID. No. 10:
ETGKKPNIWYSLNGKIRAVNLPKVTKEKPTPPVKPTAPTKPTYETEKPLKPAPV
APNYEKEPTPPTRTPDQAEPKKPTPPTYETEKPLEPAPVEPSYEAEPTPPTRTPDQAE
PNKPTPPTYETEKPLEPAPVEPSYEAEPTPPTPTPDQPEPNKPVEPTYEVIPTPPTDP
VYQDLPTPPSIPTVHFHYFKLAVQPQVNKEIRNNNDVNIDRTLVAKQSVVKFQLKTAD
LPAGRDETTSFVLVDPLPSGYQFNPEATKAASPGFDVAYDNATNTVTFKATAATLATF
NADLTKSVATIYPTVVGQVLNDGATY

Its DNA Sequence is (SEQ. ID. NO. 21):

GAAACCGGCAAAAAACCAAATATTTGGTATTCATTAAATGGTAAAATCCGTGCGGTTAAT
CTTCCTAAAGTTACTAAGGAAAAACCCACACCTCCGGTTAAACCAACAGCTCCAACTAAA
CCAACTTATGAAACAGAAAAGCCATTAAAACCGGCACCAGTAGCTCCAAATTATGAAAAG
GAGCCAACACCACCGACAAGAACACCGGATCAAGCAGAGCCAAAGAAACCCACTCCGCCG
ACCTATGAAACAGAAAAGCCGTTGGAGCCAGCACCTGTTGAGCCAAGCTATGAAGCAGAG
CCAACACCGCCGACAAGGACACCGGATCAGGCAGAGCCAAATAAACCCACACCGCCGACC
TATGAAACAGAAAAGCCGTTGGAGCCAGCACCTGTTGAGCCAAGCTATGAAGCAGAGCCA
ACGCCACCGACACCAACACCAGATCAACCAGAACCAAACAAACCTGTTGAGCCAACTTAT
GAGGTTATTCCAACACCGCCGACTGATCCTGTTTATCAAGATCTTCCAACACCTCCATCT
ATACCAACTGTTCATTTCCATTACTTTAAACTAGCTGTTCAGCCGCAGGTTAACAAAGAA
ATTAGAAACAATAACGATGTTAATATTGACAGAACTTTGGTGGCTAAACAATCTGTTGTT
AAGTTCCAGCTGAAGACAGCAGATCTCCCTGCTGGACGTGATGAAACAACTTCCTTTGTC
TTGGTAGATCCCCTGCCATCTGGTTATCAATTTAATCCTGAAGCTACAAAAGCTGCCAGC
CCTGGCTTTGATGTCGCTTATGATAATGCAACTAATACAGTCACCTTCAAGGCAACTGCA
GCAACTTTGGCTACGTTTAATGCTGATTTGACTAAGTCAGTGGCAACGATTTATCCAACA
GTGGTCGGACAAGTTCTTAATGATGGCGCAACTTAT

(xi) Residues 975 to 1004 (SEQ. ID. No. 11), which comprise a T-cell epitope.

SEQ. ID. No. 11:
QDLPTPPSIPTVHFHYFKLAVQPQVNKEIR

Its DNA Sequence is (SEQ. ID. NO. 22):
CAAGATCTTCCAACACCTCCATCTATACCAACTGTTCATTTCCATTACTTTAAACTAGCT
GTTCAGCCGCAGGTTAACAAAGAAATTAGA

The amino acid sequence of SA I/II is as follows, beginning with residue No. 1 (SEQ ID No. 23).

MKVKKTYGFRKSKISKTLCGAVLGTVAAVSVAGQKVFADETTTT
SDVDTKVVGTQTGNPATNLPEAQGSASKQAEQSQTKLERQMVHTIEVPKTDLDQAAKD
AKSAGVNVVQDADVNKGTVKTAEEAVQKETEIKEDYTKQAEDIKKTTDQYKSDVAAHE

AEVAKIKAKNQATKEQYGKDMVAHKAEVERINAANAASKTAYEAKLAQYQADLAAVQK
TNAANQASYQKALAAYQAELKRVQEANAAAKAAYDTAVAANNAKNTEIAAANEEIRKR
NATAKAEYETKLAQYQAELKRVQEANAANEADYQAKLTAYQTELARVQKANADAKAAY
EAAVAANNAKNAALTAENTAIKQRNENAKATYEAALKQYEADLAAVKKANAANEADYQ
AKLTAYQTELARVQKANADAKAAYEAAVAANNAANAALTAENTAIKKRNADAKADYEA
KLAKYQADLAKYQKDLADYPVKLKAYEDEQASIKAALAELEKHKNEDGNLTEPSAQNL
VYDLEPNANLSLTTDGKFLKASAVDDAFSKSTSKAKYDQKILQLDDLDITNLEQSNDV
ASSMELYGNFGDKAGWSTTVSNNSQVKWGSVLLERGQSATATYTNLQNSYYNGKKISK
IVYKYTVDPKSKFQGQKVWLGIFTDPTLGVFASAYTGQVEKNTSIFIKNEFTFYDEDG
KPINFDNALLSVASLNRENNSIEMAKDYTGKFVKISGSSIGEKNGMIYATDTLNFRQG
QGGARWTMYTRASEPGSGWDSSDAPNSWYGAGAIRMSGPNNSVTLGAISSTLVVPADP
TMAIETGKKPNIWYSLNGKIRAVNLPKVTKEKPTPPVKPTAPTKPTYETEKPLKPAPV
APNYEKEPTPPTRTPDQAEPKKPTPPTYETEKPLEPAPVEPSYEAEPTPPTRTPDQAE
PNKPTPPTYETEKPLEPAPVEPSYEAEPTPPTPTPDQPEPNKPVEPTYEVIPTPPTDP
VYQDLPTPPSIPTVHFHYFKLAVQPQVNKEIRNNNDVNIDRTLVAKQSVVKFQLKTAD
LPAGRDETTSFVLVDPLPSGYQFNPEATKAASPGFDVAYDNATNTVTFKATAATLATF
NADLTKSVATIYPTVVGQVLNDGATYKNNFSLTVNDAYGIKSNVVRVTTPGKPNDPDN
PNNNYIKPTKVNKNENGVVIDGKTVLAGSTNYYELTWDLDQYKNDRSSADTIQQGFYY
VDDYPEEALELRQDLVKITDANGNEVTGVSVDNYTSLEAAPQEIRDVLSKAGIRPKGA
FQIFRADNPREFYDTYVKTGIDLKIVSPMVVKKQMGQTGGSYEDQAYQIDFGNGYASN
IVINNVPKINPKKDVTLTLDPADTNNVDGQTIPLNTVFNYRLIGGIIPANHSEELFEY
NFYDDYDQTGDHYTGQYKVFAKVDITLKNGVIIKSGTELTQYTTAEVDTTKGAITIKF
KEAFLRSVSIDSAFQAESYIQMKRIAVGTFENTYINTVNGVTYSSNTVKTTTPEDPAD
PTDPQDPSSPRTSTVIIYKPQSTAYQPSSVQKTLPNTGVTNNAYMPLLGIIGLVTSFSL
LGLKAKKD

Its DNA sequence is as follows (SEQ ID No. 24):

```
  1   ATTTCAGCAA AAATTGACAA ATCAAATCAA TTATATTACA ATTTTTTAAC
 51   GTATATTACA AAAATATATT TGGAAGATTT ATTCAGATTT GGAGGATTTA
101   TGAAAGTCAA AAAAACTTAC GGTTTTCGTA AAAGTAAAAT TAGTAAAACA
151   CTGTGTGGTG CTGTTCTAGG AACAGTAGCA GCAGTCTCTG TAGCAGGACA
201   AAAGGTTTTT GCCGATGAAA CGACCACTAC TAGTGATGTA GATACTAAAG
```

```
  251  TAGTTGGAAC ACAAACTGGA AATCCAGCGA CCAATTTGCC AGAGGCTCAA
  301  GGAAGTGCGA GTAAGCAAGC TGAACAAAGT CAAACCAAGC TGGAGAGACA
  351  AATGGTTCAT ACCATTGAAG TACCTAAAAC TGATCTTGAT CAAGCAGCAA
  401  AAGATGCTAA GTCTGCTGGT GTCAATGTTG TCCAAGATGC CGATGTTAAT
  451  AAAGGAACTG TTAAAACAGC TGAAGAAGCA GTCCAAAAAG AAACTGAAAT
  501  TAAAGAAGAT TACACAAAAC AAGCTGAGGA TATTAAGAAG ACAACAGATC
  551  AATATAAATC GGATGTAGCT GCTCATGAGG CAGAAGTTGC TAAAATCAAA
  601  GCTAAAAATC AGGCAACTAA AGAACAGTAT GGAAAAGATA TGGTAGCTCA
  651  TAAAGCCGAG GTTGAACGCA TTAATGCTGC AAATGCTGCC AGTAAAACAG
  701  CTTATGAAGC TAAATTGGCT CAATATCAAG CAGATTTAGC AGCCGTTCAA
  751  AAAACCAATG CTGCCAATCA AGCATCCTAT CAAAAAGCCC TTGCTGCTTA
  801  TCAGGCTGAA CTGAAACGTG TTCAGGAAGC TAATGCAGCC GCCAAAGCCG
  851  CTTATGATAC TGCTGTAGCA GCAAATAATG CCAAAAATAC AGAAATTGCC
  901  GCTGCCAATG AAGAAATTAG AAAACGCAAT GCAACGGCCA AAGCTGAATA
  951  TGAGACTAAG TTAGCTCAAT ATCAAGCTGA ACTAAAGCGT GTTCAGGAAG
 1001  CTAATGCCGC AAACGAAGCA GACTATCAAG CTAAATTGAC CGCCTATCAA
 1051  ACAGAGCTTG CTCGCGTTCA GAAAGCCAAT GCAGATGCTA AAGCGGCCTA
 1101  TGAAGCAGCT GTAGCAGCAA ATAATGCCAA AAATGCGGCA CTTACAGCTG
 1151  AAAATACTGC AATTAAGCAA CGCAATGAGA ATGCTAAGGC GACTTATGAA
 1201  GCTGCACTCA AGCAATATGA GGCTGATTTG GCAGCGGTGA AAAAAGCTAA
 1251  TGCCGCAAAC GAAGCAGACT ATCAAGCTAA ATTGACCGCC TATCAAACAG
 1301  AGCTCGCTCG CGTTCAAAAG GCCAATGCGG ATGCTAAAGC GGCCTATGAA
 1351  GCAGCTGTAG CAGCAAATAA TGCCGCAAAT GCAGCGCTCA CAGCTGAAAA
 1401  TACTGCAATT AAGAAGCGCA ATGCGGATGC TAAAGCTGAT TACGAAGCAA
 1451  AACTTGCTAA GTATCAAGCA GATCTTGCCA AATATCAAAA AGATTTAGCA
 1501  GACTATCCAG TTAAGTTAAA GGCATACGAA GATGAACAAG CTTCTATTAA
 1551  AGCTGCACTG GCAGAACTTG AAAAACATAA AAATGAAGAC GGAAACTTAA
 1601  CAGAACCATC TGCTCAAAAT TTGGTCTATG ATCTTGAGCC AAATGCGAAC
 1651  TTATCTTTGA CAACAGATGG GAAGTTCCTT AAGGCTTCTG CTGTGGATGA
 1701  TGCTTTTAGC AAAAGCACTT CAAAAGCAAA ATATGACCAA AAAATTCTTC
 1751  AATTAGATGA TCTAGATATC ACTAACTTAG AACAATCTAA TGATGTTGCT
 1801  TCTTCTATGG AGCTTTATGG CAATTTTGGT GATAAAGCTG GCTGGTCAAC
 1851  GACAGTAAGC AATAACTCAC AGGTTAAATG GGGATCGGTA CTTTTAGAGC
```

1901 GCGGTCAAAG CGCAACAGCT ACATACACTA ACCTGCAGAA TTCTTATTAC
2001 GTCCAAGTTT CAAGGTCAAA AGGTTTGGTT AGGTATTTTT ACCGATCCAA
1951 AATGGTAAAA AGATTTCTAA AATTGTCTAC AAGTATACAG TGGACCCTAA
2051 CTTTAGGTGT TTTTGCTTCC GCTTATACAG GTCAAGTTGA AAAAAACACT
2101 TCTATTTTTA TTAAAAATGA ATTCACTTTC TATGACGAAG ATGGAAAACC
2151 AATTAATTTT GATAATGCCC TTCTATCAGT AGCTTCTCTT AACCGAGAAA
2201 ATAATTCTAT TGAGATGGCC AAAGATTATA CGGGTAAATT TGTCAAAATC
2251 TCTGGATCAT CTATCGGTGA AAAGAATGGC ATGATTTATG CTACAGATAC
2301 TCTCAACTTT AGGCAGGGTC AAGGTGGTGC TCGTTGGACC ATGTATACCA
2351 GAGCTAGCGA ACCGGGATCT GGCTGGGATA GTTCAGATGC GCCTAACTCT
2401 TGGTATGGTG CTGGTGCTAT CCGCATGTCT GGTCCTAATA ACAGTGTGAC
2451 TTTGGGTGCT ATCTCATCAA CACTTGTTGT GCCTGCTGAT CCTACAATGG
2501 CAATTGAAAC CGGCAAAAAA CCAAATATTT GGTATTCATT AAATGGTAAA
2551 ATCCGTGCGG TTAATCTTCC TAAAGTTACT AAGGAAAAAC CCACACCTCC
2601 GGTTAAACCA ACAGCTCCAA CTAAACCAAC TTATGAAACA GAAAAGCCAT
2651 TAAAACCGGC ACCAGTAGCT CCAAATTATG AAAAGGAGCC AACACCACCG
2701 ACAAGAACAC CGGATCAAGC AGAGCCAAAG AAACCCACTC CGCCGACCTA
2751 TGAAACAGAA AAGCCGTTGG AGCCAGCACC TGTTGAGCCA AGCTATGAAG
2801 CAGAGCCAAC ACCGCCGACA AGGACACCGG ATCAGGCAGA GCCAAATAAA
2851 CCCACACCGC CGACCTATGA AACAGAAAAG CCGTTGGAGC CAGCACCTGT
2901 TGAGCCAAGC TATGAAGCAG AGCCAACGCC ACCGACACCA ACACCAGATC
2951 AACCAGAACC AAACAAACCT GTTGAGCCAA CTTATGAGGT TATTCCAACA
3001 CCGCCGACTG ATCCTGTTTA TCAAGATCTT CCAACACCTC CATCTATACC
3051 AACTGTTCAT TTCCATTACT TTAAACTAGC TGTTCAGCCG CAGGTTAACA
3101 AAGAAATTAG AAACAATAAC GATGTTAATA TTGACAGAAC TTTGGTGGCT
3151 AAACAATCTG TTGTTAAGTT CCAGCTGAAG ACAGCAGATC TCCCTGCTGG
3201 ACGTGATGAA ACAACTTCCT TTGTCTTGGT AGATCCCCTG CCATCTGGTT
3251 ATCAATTTAA TCCTGAAGCT ACAAAAGCTG CCAGCCCTGG CTTTGATGTC
3301 GCTTATGATA ATGCAACTAA TACAGTCACC TTCAAGGCAA CTGCAGCAAC
3351 TTTGGCTACG TTTAATGCTG ATTTGACTAA GTCAGTGGCA ACGATTTATC
3401 CAACAGTGGT CGGACAAGTT CTTAATGATG GCGCAACTTA TAAGAATAAT
3451 TTCTCGCTCA CAGTCAATGA TGCTTATGGC ATTAAATCCA ATGTTGTTCG
3501 GGTGACAACT CCTGGTAAAC CAAATGATCC AGATAACCCA AATAATAATT

3551 ACATTAAGCC AACTAAGGTT AATAAAAATG AAAATGGCGT TGTTATTGAT
3601 GGTAAAACAG TTCTTGCCGG TTCAACGAAT TATTATGAGC TAACTTGGGA
3651 TTTGGATCAA TATAAAAACG ACCGCTCTTC AGCAGATACC ATTCAACAAG
3701 GATTTTACTA TGTAGATGAT TATCCAGAAG AAGCGCTTGA ATTGCGTCAG
3751 GATTTAGTGA AGATTACAGA TGCTAATGGC AATGAAGTTA CTGGTGTTAG
3801 TGTGGATAAT TATACTAGTC TTGAAGCAGC CCCTCAAGAA ATTAGAGATG
3851 TTCTTTCTAA GGCAGGAATT AGACCTAAAG GTGCTTTCCA AATTTTCCGT
3901 GCCGATAATC CAAGAGAATT TTATGATACT TATGTCAAAA CTGGAATTGA
3951 TTTGAAGATT GTATCACCAA TGGTTGTTAA AAAACAAATG GGACAAACAG
4001 GCGGGAGTTA TGAAGATCAA GCTTACCAAA TTGACTTTGG TAATGGTTAT
4051 GCATCAAATA TCGTTATCAA TAATGTTCCT AAGATTAACC CTAAGAAAGA
4101 TGTGACCTTA ACACTTGATC CGGCTGATAC AAATAATGTT GATGGTCAGA
4151 CTATTCCACT TAATACAGTC TTTAATTACC GTTTGATTGG TGGCATTATC
4201 CCTGCAAATC ACTCAGAAGA ACTCTTTGAA TACAATTTCT ATGATGATTA
4251 TGATCAAACA GGAGATCACT ATACTGGTCA GTATAAAGTT TTTGCCAAGG
4301 TTGATATCAC TCTTAAAAAC GGTGTTATTA TCAAGTCAGG TACTGAGTTA
4351 ACTCAGTATA CGACAGCGGA AGTTGATACC ACTAAAGGTG CTATCACAAT
4401 TAAGTTCAAG GAAGCCTTTC TGCGTTCTGT TTCAATTGAT TCAGCCTTCC
4451 AAGCTGAAAG TTATATCCAA ATGAAACGTA TTGCGGTTGG TACTTTTGAA
4501 AATACCTATA TTAATACTGT CAATGGGGTA ACTTACAGTT CAAATACAGT
4551 GAAAACAACT ACTCCTGAGG ATCCTGCAGA CCCTACTGAT CCGCAAGATC
4601 CATCATCACC GCGGACTTCA ACTGTAATTA TCTACAAACC TCAATCAACT
4651 GCTTATCAAC CAAGCTCTGT CCAAAAAACG TTACCAAATA CGGGAGTAAC
4701 AAACAATGCT TATATGCCTT TACTTGGTAT TATTGGCTTA GTTACTAGTT
4751 TTAGTTTGCT TGGCTTAAAG GCTAAGAAAG ATTGACAGCA TAGATATTAC
4801 ATTAGAATTA AAAAGTGAGA TGAAGCGATA AATCACAGAT TGAGCTTTTA
4851 TCTCATTTTT TGATT

CLAIMS

1. A nucleic acid sequence which codes upon expression in a prokaryotic or eukaryotic host cell for a polypeptide product having one or more properties selected from (i) the ability to adhere to a mammalian tooth in a competitive manner with naturally occurring Streptococcus mutans antigen I/II, thus preventing or diminishing the adhesion of S.mutans to the tooth; (ii) the ability to stimulate a T-cell response; and (iii) the ability to stimulate a B-cell response, said nucleic acid sequence being selected from:

(a) the sequences shown in SEQ. ID. No. 12 to 22 or the complementary strands thereof;

(b) nucleic acid sequences having a length of not more than 1000 base pairs which hybridise to the sequences defined in (a) over at least 70% of their length;

(c) nucleic acid sequences having a length of not more than 1000 base pairs which, but for the degeneracy of the genetic code, would hybridise to the nucleic acid sequences defined in (a) or (b) over at least 70% of their length and which sequences code for polypeptides having the same amino acid sequence.

2. A nucleic acid sequence as defined in claim 1 which is extended at either or both of the 5' and 3' ends by non-wild-type nucleic acid sequence.

3. A polypeptide encoded by a nucleic acid sequence as defined in claim 1 or 2.

4. A polypeptide according to claim 3 comprising a

sequence as shown in any one of SEQ. ID. No. 1 to 11.

5. A vector comprising a nucleic acid sequence according to claim 1 or 2 operably linked to a promoter capable of directing its expression in a host cell.

6. A Cell harbouring a vector according to claim 5.

7. A pharmaceutical composition comprising a polypeptide according to claim 3 or 4 and a pharmaceutically acceptable carrier.

8. A composition comprising according to claim 7 that is a vaccine composition.

9. A polypeptide according to claim 3 or 4 for use in the treatment of dental caries.

10. A method of vaccinating a mammalian host against dental caries or treating dental caries, which method comprises administering to the host an effective amount of a polypeptide according to claim 3 or 4 or a composition according to claim 8 or 9.

Fig.1.

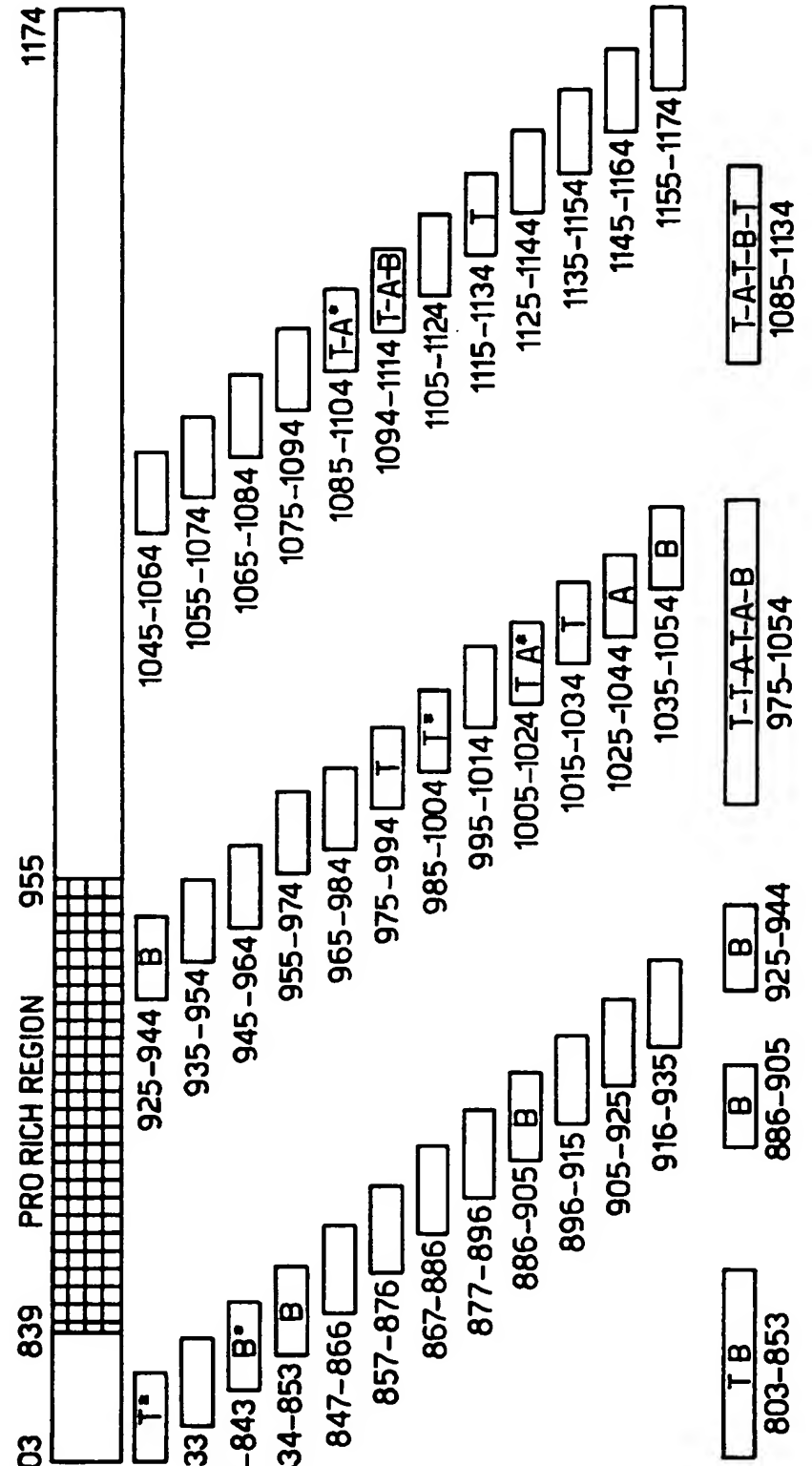
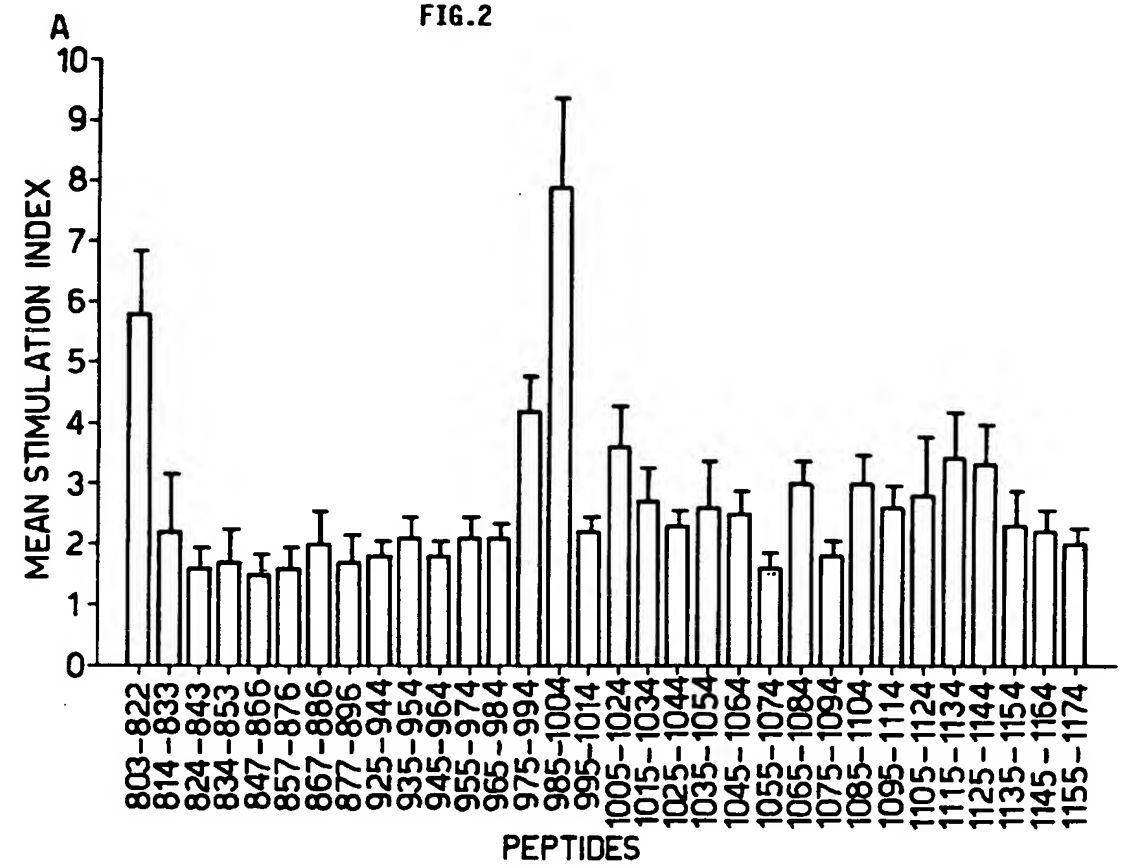

803
839
PRO RICH REGION
955
1174
803–822 T*
814–833
824–843 B*
834–853 B
847–866
857–876
867–886
877–896
886–905 B
896–915
905–925
916–935
925–944 B
935–954
945–964
955–974
965–984
975–994 T
985–1004 T*
995–1014
1005–1024 T A*
1015–1034 T
1025–1044 A
1035–1054 B
1045–1064
1055–1074
1065–1084
1075–1094
1085–1104 T-A*
1094–1114 T-A-B
1105–1124
1115–1134 T
1125–1144
1135–1154
1145–1164
1155–1174
T B
803–853
B
886–905
B
925–944
T-T-A-T-A-B
975–1054
T-A-T-B-T
1085–1134

FIG.2


A
MEAN STIMULATION INDEX
0
1
2
3
4
5
6
7
8
9
10
803-822
814-833
824-843
834-853
847-866
857-876
867-886
877-896
925-944
935-954
945-964
955-974
965-984
975-994
985-1004
995-1014
1005-1024
1015-1034
1025-1044
1035-1054
1045-1064
1055-1074
1065-1084
1075-1094
1085-1104
1095-1114
1105-1124
1115-1134
1125-1144
1135-1154
1145-1164
1155-1174
PEPTIDES

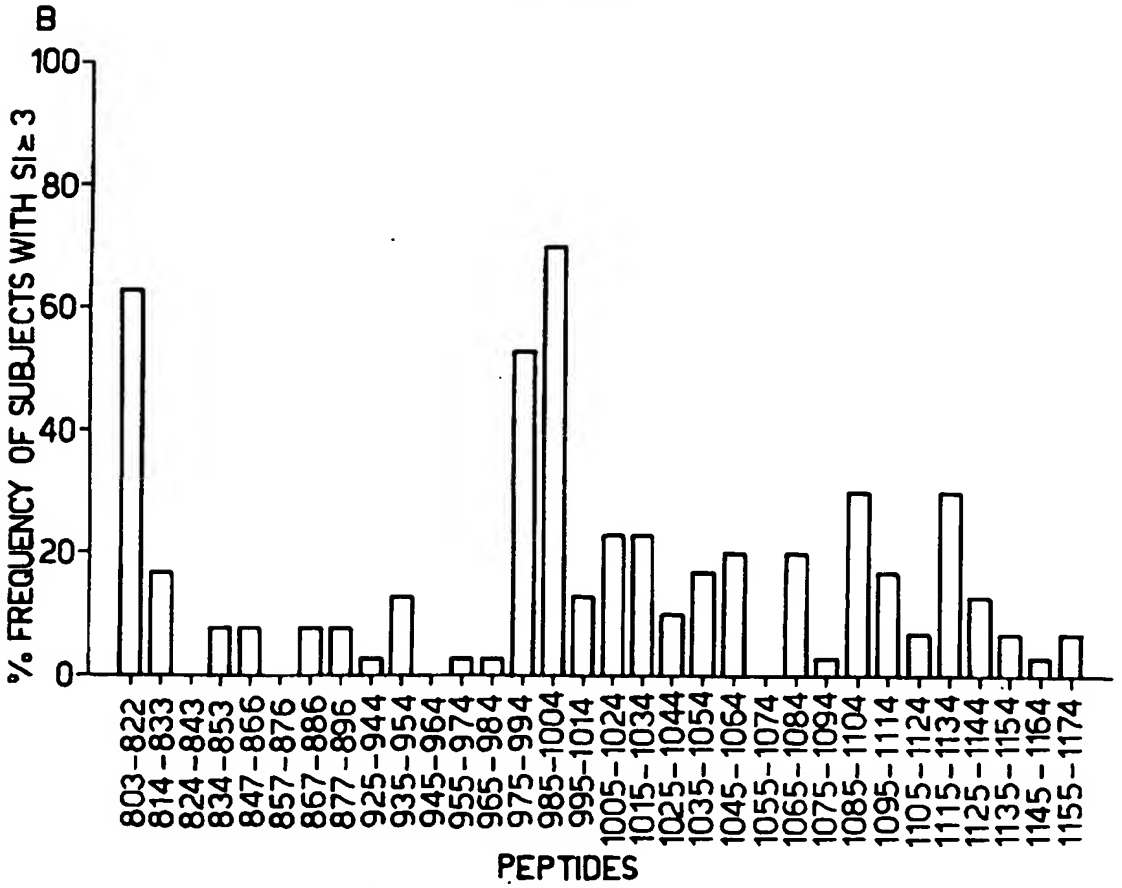

B
% FREQUENCY OF SUBJECTS WITH SI≥3
0
20
40
60
80
100
803-822
814-833
824-843
834-853
847-866
857-876
867-886
877-896
925-944
935-954
945-964
955-974
965-984
975-994
985-1004
995-1014
1005-1024
1015-1034
1025-1044
1035-1054
1045-1064
1055-1074
1065-1084
1075-1094
1085-1104
1095-1114
1105-1124
1115-1134
1125-1144
1135-1154
1145-1164
1155-1174
PEPTIDES

Fig.3.

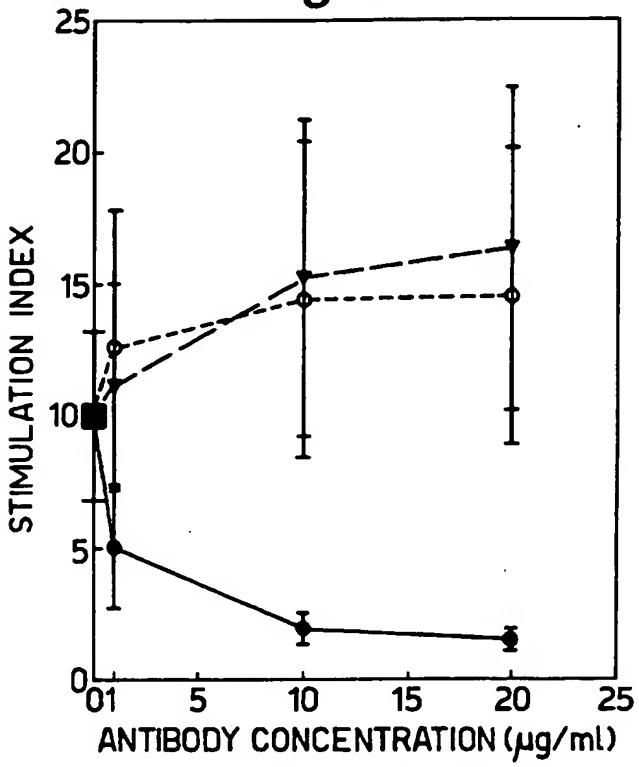

25
20
15
10
5
0
STIMULATION INDEX
01
5
10
15
20
25
ANTIBODY CONCENTRATION (µg/ml)

Fig.4.

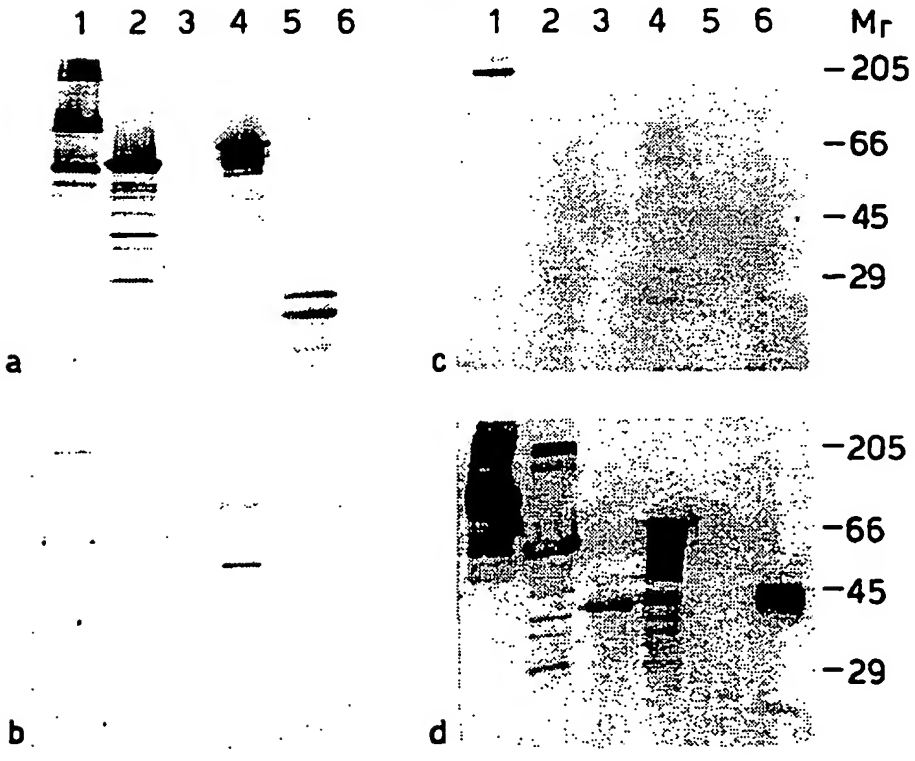

1 2 3 4 5 6
1 2 3 4 5 6
Mr
205
66
45
29
a
c
205
66
45
29
b
d

Fig.5.

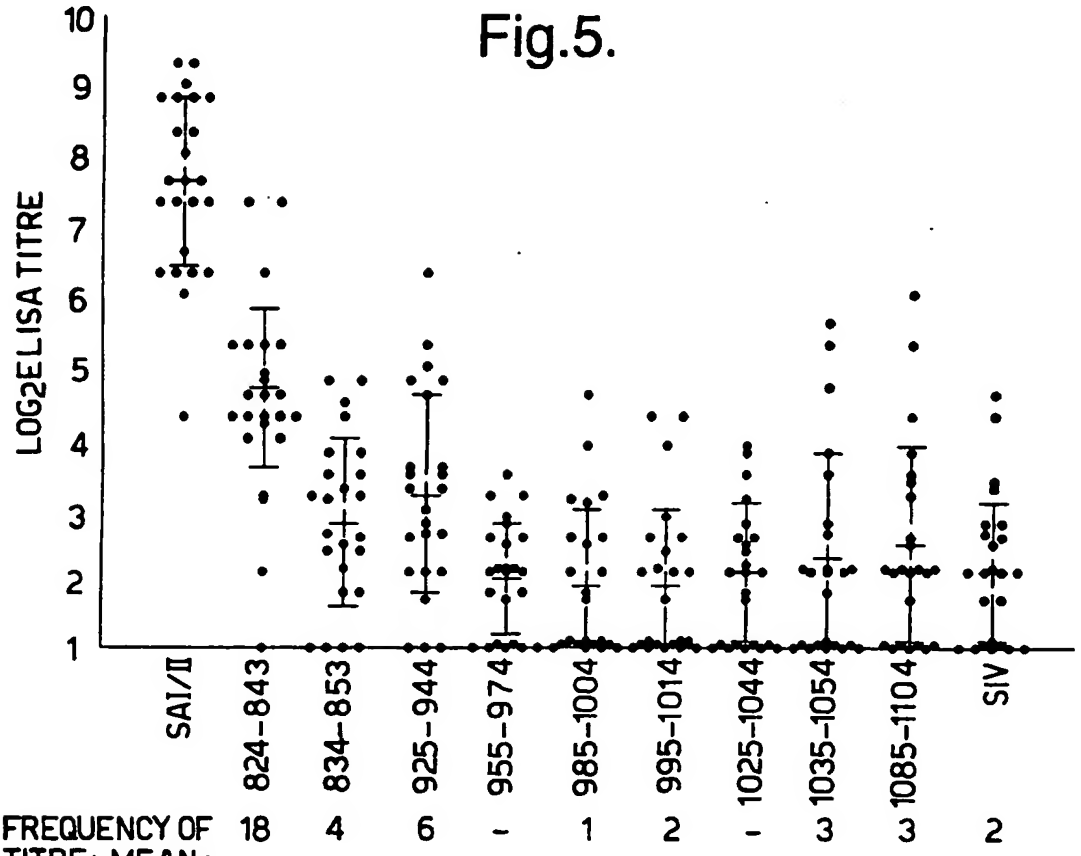

LOG2ELISA TITRE
10
9
8
7
6
5
4
3
2
1
SAI/II
824-843
834-853
925-944
955-974
985-1004
995-1014
1025-1044
1035-1054
1085-1104
SIV
FREQUENCY OF TITRE≥MEAN+ 2SD OF SIV PEPTIDE (n = 22)
18
4
6
1
1
2
1
3
3
2

Fig.7.

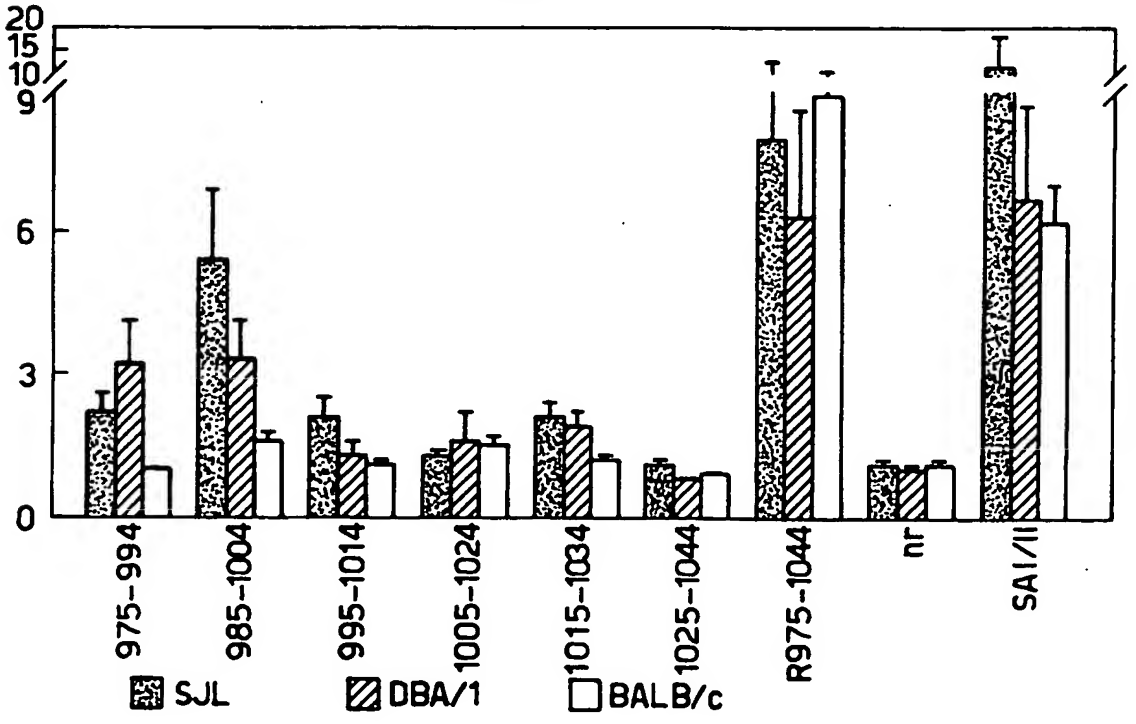
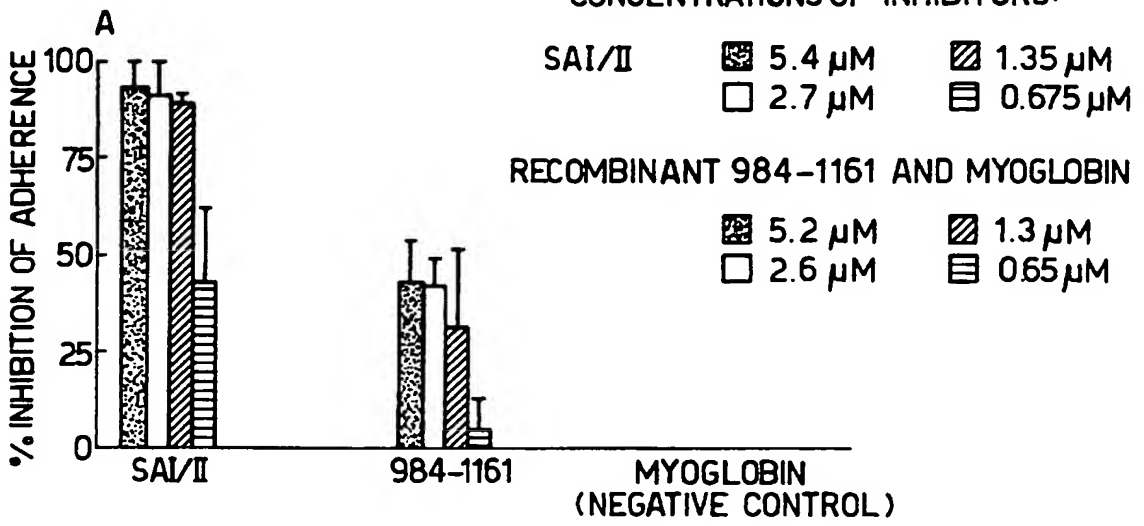

20
15
10
9
6
3
0
975-994
985-1004
995-1014
1005-1024
1015-1034
1025-1044
R975-1044
nr
SAI/II
SJL
DBA/1
BALB/c

FIG.6


RESULTS ARE MEAN ± SD FROM 3 ASSAYS.
CONCENTRATIONS OF INHIBITORS:
SAI/II
5.4 μM
1.35 μM
2.7 μM
0.675 μM
RECOMBINANT 984-1161 AND MYOGLOBIN
5.2 μM
1.3 μM
2.6 μM
0.65 μM
A
% INHIBITION OF ADHERENCE
100
75
50
25
0
SAI/II
984-1161
MYOGLOBIN
(NEGATIVE CONTROL)

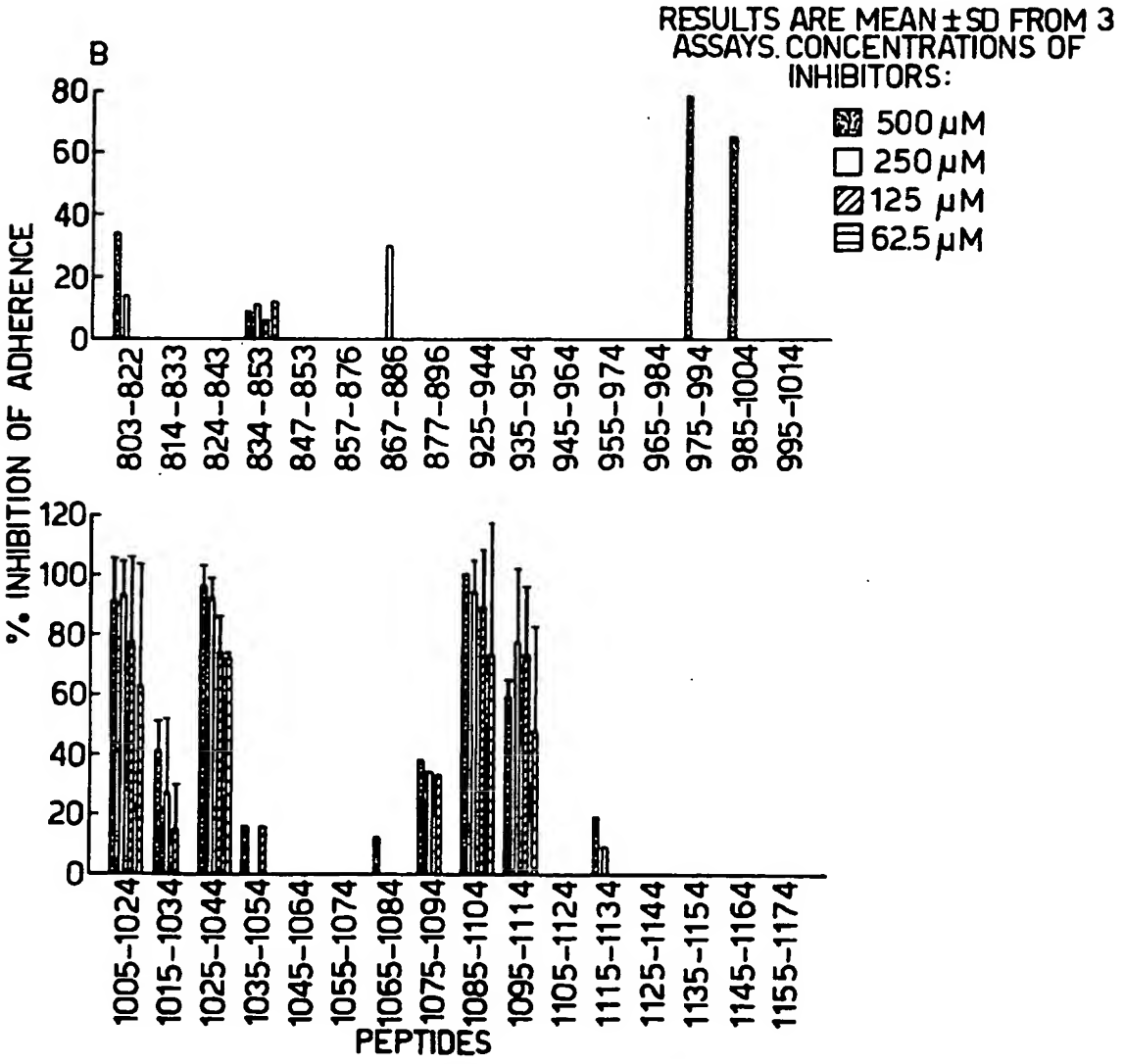

RESULTS ARE MEAN ± SD FROM 3 ASSAYS. CONCENTRATIONS OF INHIBITORS:
500 μM
250 μM
125 μM
62.5 μM
B
% INHIBITION OF ADHERENCE
80
60
40
20
0
803-822
814-833
824-843
834-853
847-853
857-876
867-886
877-896
925-944
935-954
945-964
955-974
965-984
975-994
985-1004
995-1014
120
100
80
60
40
20
0
1005-1024
1015-1034
1025-1044
1035-1054
1045-1064
1055-1074
1065-1084
1075-1094
1085-1104
1095-1114
1105-1124
1115-1134
1125-1144
1135-1154
1145-1164
1155-1174
PEPTIDES

Fig.8.

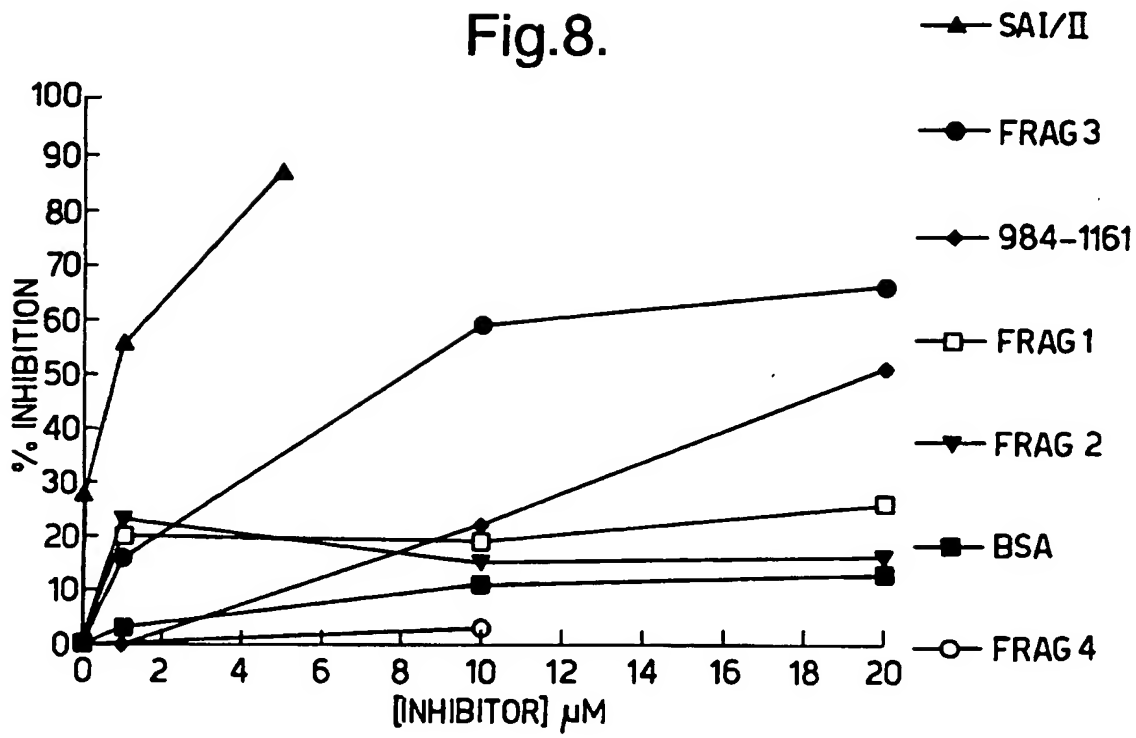

SAI/II
FRAG 3
984-1161
FRAG 1
FRAG 2
BSA
FRAG 4
% INHIBITION
[INHIBITOR] μM
0
10
20
30
40
50
60
70
80
90
100
0
2
4
6
8
10
12
14
16
18
20

Fig.9.

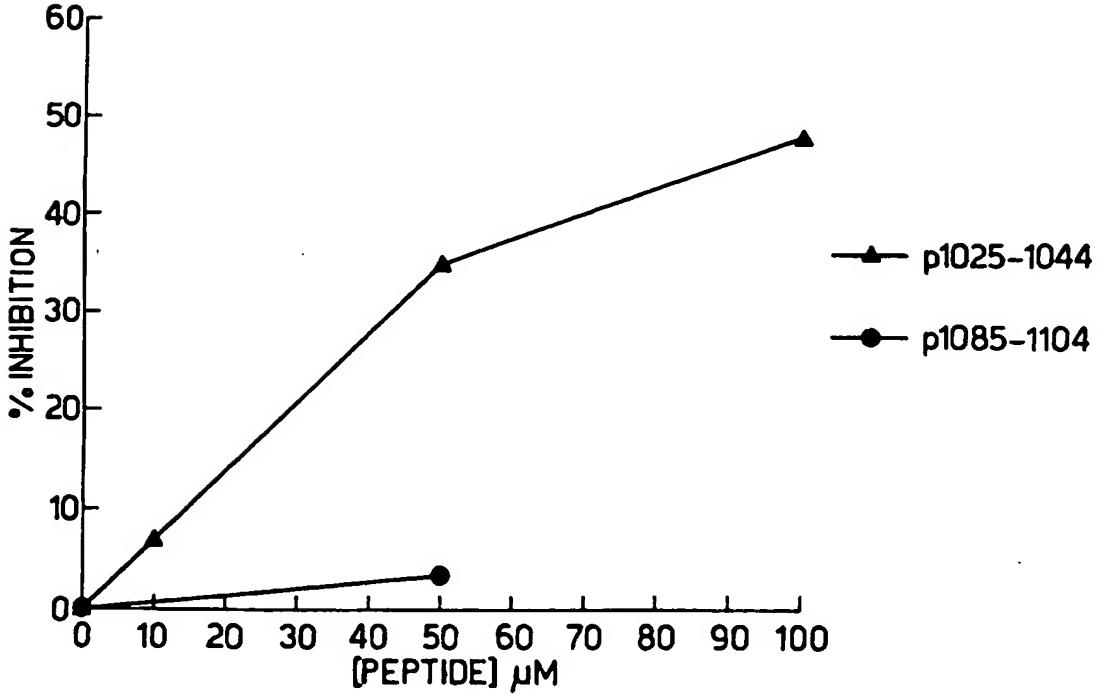

p1025-1044
p1085-1104
% INHIBITION
[PEPTIDE] μM
0
10
20
30
40
50
60
0
10
20
30
40
50
60
70
80
90
100

Fig.10.

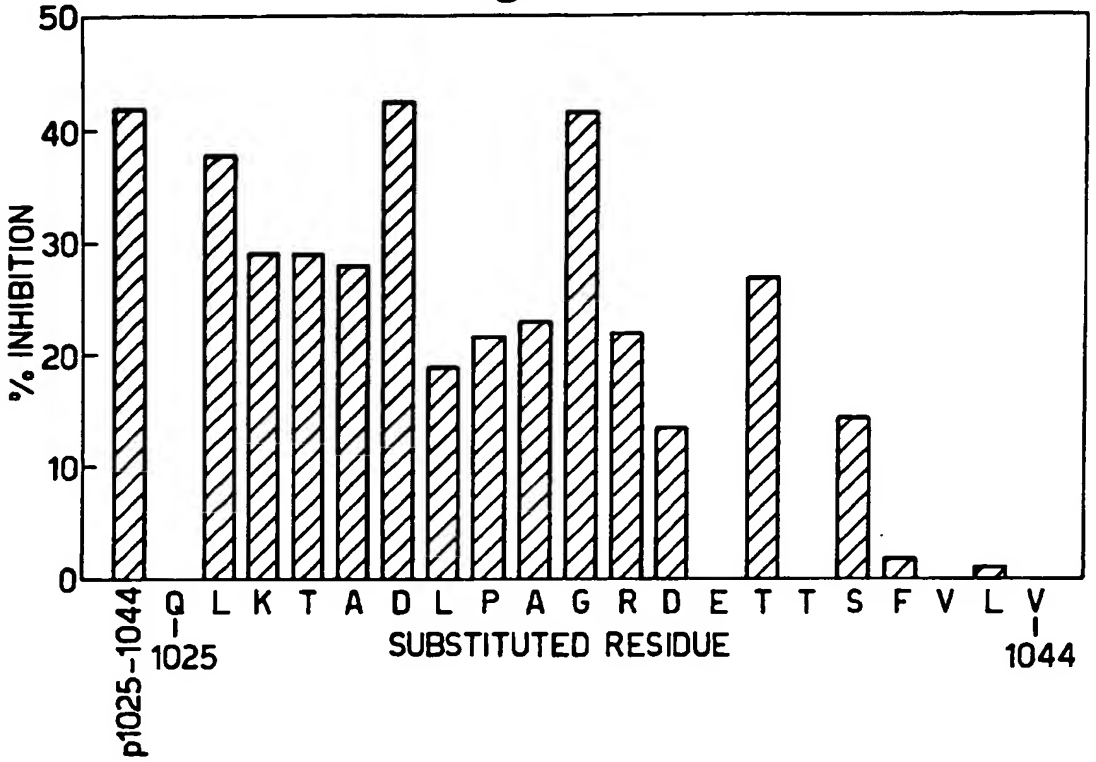
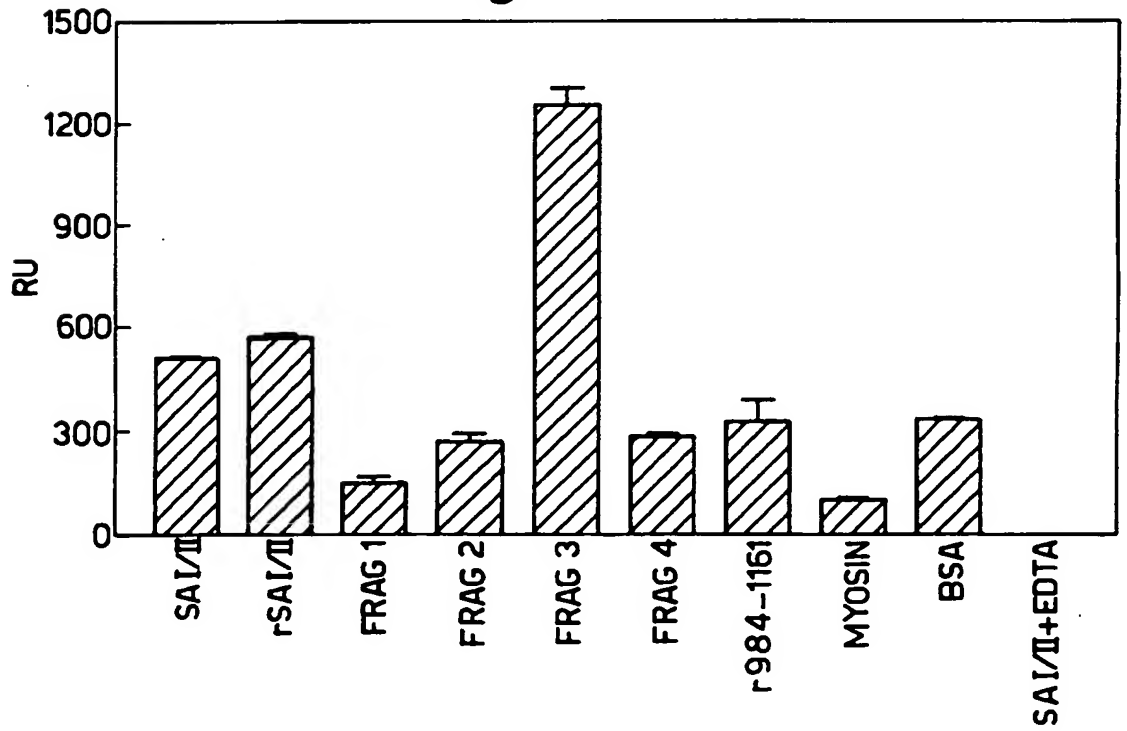

50
40
30
20
10
0
% INHIBITION
p1025-1044
Q
1025
L K T A D L P A G R D E T T S F V L V
SUBSTITUTED RESIDUE
1044

Fig.11.


1500
1200
900
600
300
0
RU
SAI/II
rSAI/II
FRAG 1
FRAG 2
FRAG 3
FRAG 4
r984-1161
MYOSIN
BSA
SAI/II+EDTA

INTERNATIONAL SEARCH REPORT

Int ional Application No
PCT/GB 96/00207

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/31 C07K14/315 A61K39/09 //C12N15/62

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07K C12N A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	INFECTION AND IMMUNITY, (1994 JAN) 62 (1) 184-93, XP002003240 MOISSET, A. ET AL.: "Conservation of salivary glycoprotein-interacting and human immunoglobulin G-cross-reactive domains of antigen I / II in oral streptococci."	3,9,11
Y	see table 2 ---	1-9,11
Y	INFECTION AND IMMUNITY, (1993 NOV) 61 (11) 4590-8, XP002003241 MUNRO, G. ET AL.: "A protein fragment of streptococcal cell surface antigen I / II which prevents adhesion of Streptococcus mutans." see the whole document --- -/--	1-9,11

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

1

Date of the actual completion of the international search	Date of mailing of the international search report
20 May 1996	17.06.96
Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+ 31-70) 340-3016	Authorized officer Andres, S

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB 96/00207

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	EP,A,0 280 576 (ST THOMAS HOSP MED SCHOOL ;JOHNSON & JOHNSON CONSUM PROD (US)) 31 August 1988 see the whole document	1-9,11
O,A	J.DENT.RES. 71 (IADR ABSTRACTS); 1992, 735, ABSTRACT 1753, XP002003347 MUNRO, G. ET AL.: "MAPPING OF ADHESION EPITOPES OF STREPTOCOCCAL ANTIGEN I - II." see abstract	1-9,11
O,A	J.DENT.RES. 72 (SPECIAL ISSUE; IADR ABSTRACTS), 1992, 517, ABSTRACT 17, XP002003242 KELLY, C. ET AL.: "GENETIC AND IMMUNOLOGICAL ANALYSIS OF CONSERVED EPITOPES OF ANTIGEN I - II IN ORAL STREPTOCOCCI." see abstract	1-6
A	GB,A,2 060 647 (RUSSELL M W;LEHNER T) 7 May 1981 cited in the application	
P,X	INFECTION AND IMMUNITY, (1995 SEP) 63 (9) 3649-58, XP002003243 KELLY, C. ET AL.: "T-cell, adhesion, and B-cell epitopes of the cell surface Streptococcus mutans protein antigen I/II." see the whole document	1-9,11

1

INTERNATIONAL SEARCH REPORT

Information on patent family members

Ir tional Application No
PCT/GB 96/00207

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
EP-A-0280576	31-08-88	AU-B-	1349588	26-09-88
		EP-A-	0346370	20-12-89
		WO-A-	8806594	07-09-88
		JP-T-	2502637	23-08-90
		ZA-A-	8801337	26-08-88
GB-A-2060647	07-05-81	WO-A-	8101101	30-04-81